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# CONTRIBUTION TO THE CHEMICAL TAXONOMY OF THE AGARICALES PIGMENTS OF BOLETUS AND CORTINARIA

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# CONTRIBUTION TO THE CHEMICAL TAXONOMY OF THE AGARICALES
# PIGMENTS OF BOLETUS AND CORTINARIA *

M. Gabriel

It would have been impossible for me to undertake this work and /7*/** to successfully carry it out without the support granted me by the National Center for Scientific Research, first as a trainee and later as research assistant.

This research was carried out at the General Botany Laboratory, where Dean R. Douin welcomed me, and earned my profound gratitude; I deeply regret that his brutal death did not allow him to see this study concluded.

The subject of this work was suggested by professor R. Kühner, who should see here an expression of my respectful gratitude for giving me the benefit of his great knowledge. Thanks to him, the interest I brought to this subject has only grown. All during this research he has always generously given of his advice and criticism. I am very grateful to him for the numerous determinations of species he performed, and for the readiness with which he made his unpublished notes on the microtopography of pigmentation

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** Numbers on the margin indicate pagination of the original foreign text.

available to me.

My gratitude is equally directed towards Mr. P. Lebreton, the lecturer who let me profit from his vast experience in the realm of chemistry, and whose advice has been so useful to me.

I thank Professor J. Boidin who was kind enough to do me the honor of being part of my thesis examining board, and who has given sound counsel on many occasions.

Thanks to the kindness of Professor J. Lichtenberger I had access to a number of chemical samples, for which I am respectfully grateful.

I deeply thank Miss D. Lamoure, Assistant teacher, for her interest in the work and for obtaining many species for me.

I do not want to forget Mr. A. Pouchet, the lamented President, nor Mr. M. Josserand, of the Linnean Society of Lyon, who have determined and obtained several species for me.

It is a pleasure to thank all my colleagues at the Botany and Microbiology Laboratories for their frequent assistance and the kindness they have always shown me.

## INTRODUCTION

Mushrooms are a vegetable group extraordinarily rich in pigments. Among superior mushrooms the pigments are essentially limited to the carpophores. There are great differences in shade from one species to another, and even among different parts of a given carpophore; also color has always held an important place among the characteristics used to distinguish and classify the species. As an example: it is the difference in the coloration of the carpophores (fruit bodies) that has led to recognition of the heterogeneity of the groups Stropharia, Hypholoma and Psilocybe and regrouping the members in two much more natural genera: Geophila and Drosophila. Let us recall also the role spore color played in the classical mushroom system elaborated by E. Fries.

Early authors limited themselves to observing the colors, but later the microscopic aspect of the pigmentation was also taken into account. R. Kühner has called attention to significant differences in the pigment topography of mushroom carpophores in 1934, foretelling differences of a chemical order and showing the systematic interest of these observations.

Besides the information obtained from a simple color examination, mycologists have for a long time used several chemical reactions to aid in the characterization of a species (Batelle, 1948; Henry 1943). Doubtlessly many of these reactions are a result of the transformation of colored substances, and they depend on their chemical nature.

It is certain that some research on the structure of pigments will be a great help to the systematician, and thus the pigmentation criterion will take on its full value. R. Heim (1942) in a remarkable conference on the taxonomic importance of pigments in mushrooms, rightly pointed out the fragility of an essentially

subjective criterion if not supported by a physico-chemical study.

The strictly chemical study of pigments was particularly developed in the case of some groups of imperfect mushrooms (Helminthosporium - Aspergillaceae) and the results have confirmed and made more precise the generic results of the systematicians; this is far from being the case with the higher fungi, where chemical research remains fragmentary and never was oriented taxonomically.

## HISTORICAL ACCOUNT

For a detailed historical account of the pigments of mushrooms, I refer the reader to the review articles by Pastac (1942), Bonnet (1959) and Hegnauer (1962).

Below we simply deal with some pigments identified in mushrooms. The Boletus are included in this order, but Chanterelles are excluded, particularly because several of them have, in high concentration, some carotenoid pigments, while no pigment of this chemical family has ever been found in Agaricus mushrooms.

ATROMENTINE: di(p-hydroxyphenyl)-2,5 dihydroxy-3,6 benzoquinone
isolated by Thörner (1879) from Paxillus atrotomentosus; formula obtained by Kögl and Postowsky (1924 and 1925b), synthesis performed by Kögl and Becker (1928).

EMODINE: tetrahydroxy-4,5,7,8 methoxy-6 methyl-2 anthraquinone or tetra hydroxy-4,5,6,8 methoxy-7 methyl-2 anthraquinone isolated from Cortinarius sanguineus, empirical formula rendered by Kögl and Postowsky (1925a), definitive formula and synthesis by Birkinshaw and Gourlay (1961).

MUSCARUFINE: hydroxy-6( $\delta$ carboxy, $\alpha\gamma$ butadienyl)-3 di(o-carboxyphenyl)-2,5 benzoquinone
isolated from Amanita muscaria, formula obtained by Kögl and Erxleben (1930)

BOLETOL: trihydroxy-1,2,4 carboxy-5 or 8 anthraquinone
isolated from several species of Boletus by G. Bertrand (1902), formula and synthesis by Kögl and Delis (1935)

LACTAROVIOLINE: formyl-1 methyl-4 isopropenyl-7 azulene
isolated from Lactarius deliciosus by Willstaedt (1935-

1936), formula obtained by Plattner et al. (1954).

LACTARAZULENE: dimethyl-1,4 isopropenyl-7 azulene /12
isolated from Lactarius deliciosus by Willstaedt (1935-1936), formula obtained by Benesova et al. (1954-1955).

VERDAZULENE: $C_{15}H_{16}$, structural formula unknown
isolated by Willstaedt (1935-1936).

It seemed convenient to me to start my research with groups for which the chemical constitution of the pigments was known for some species, such as Boletus and Cortinata. The study of the latter genus was particularly necessary because is the widest of the genera of Agaricus mushrooms and one whose species present a wide variety of colors. Besides, one frequently resorts to color reactions for the determinations and, last of all, according to R. Kühner, the microtopography is varied.

Among the two genera, anthraquinone pigments were the most frequently found: anthraquinones and anthranoles, and I have identified, in a group of Cortinata, a xanthone pigment, a type of pigment until then found only in the lower mushrooms. Prior to relating my personal research, I find it useful to review the chemistry of these pigments.

# 1. ANTHRAQUINONES AND ANTHRANOLS.

The works of Y. Asahina and S. Shibata (1954), B.-H. Howard and H. Raistrick, (1955) and R.-H. Thomson (1957) have been invaluable in the preparation of this review.

## 1.1. Formulation

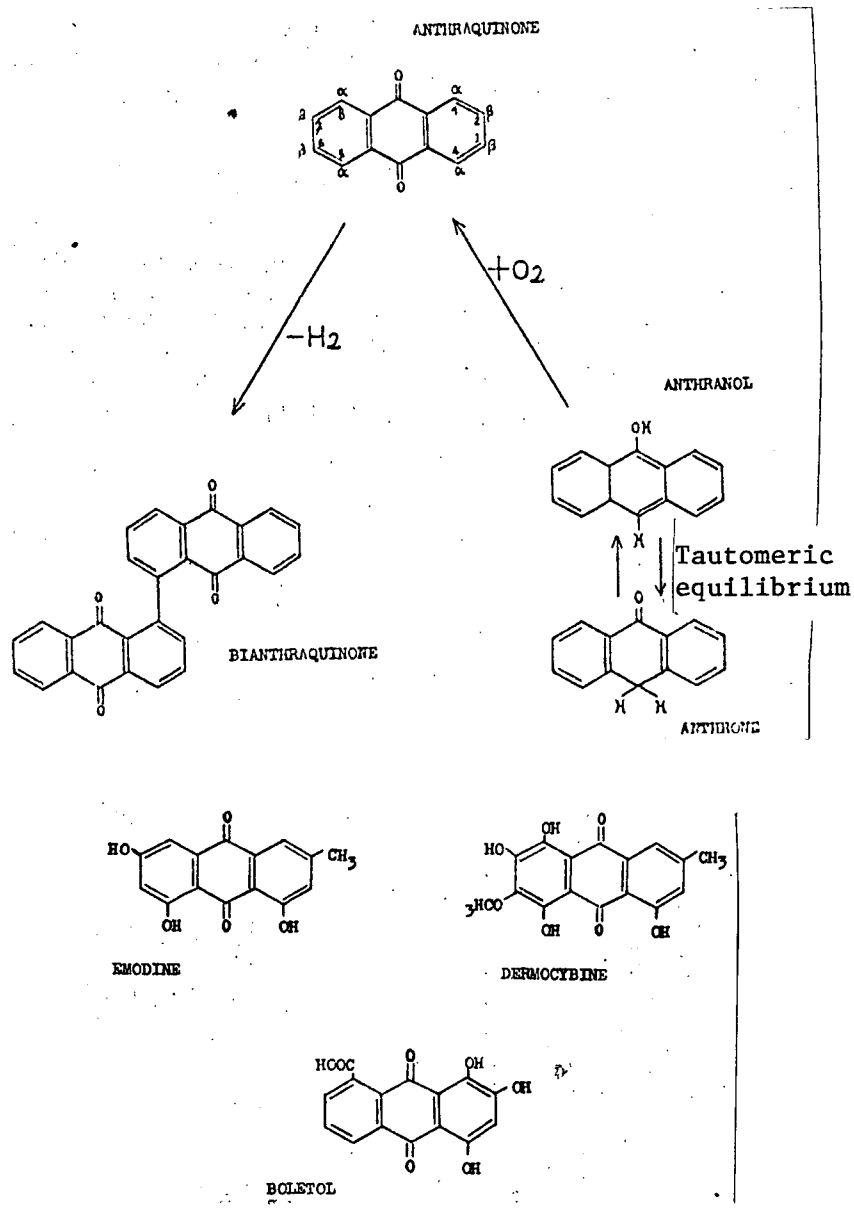
Two structural groups can be placed next to anthraquinones, with the same skeleton (anthracene) and derived from anthraquinones either by reduction (anthranoles, anthranones) or by oxidation (biantraquinones).

The oxidation of anthranols to anthraquinones can be readily achieved by means of hydrogen peroxide in an alkaline medium. In the presence of sodium hydrosulphite, bianthraquinones decompose into anthraquinones.

The most frequent substitutes on a natural anthraquinone nucleus /14 are the -OH groups. They can occupy two different positions with respect to the =CO of the quinone: α position (at 1,4,5, or 8) in which the -OH group is still close to a quinone =CO with which it forms hydrogen bonds; or β position (at 2,3,6 and 7). The color of the anthraquinones, their solubility, their color reactions and their spectra are obviously influenced by the number of substitutions, but also by their position, as we shall see later.

Natural anthraquinones, moreover, very often have the substitutes $-CH_3$, $-CH_2OH$, -COOH in small numbers and, sometimes although not often, a longer or shorter carbon side chain. These substitutes are usually in a β position.

Let us point out, with regard to boletol, that the position of




the -COOH group has not been precisely established and that it is either 5 or 8; in one case it is boletol and in the other an iso-boletol obtained together with boletol during synthesis.

## 1.2. SOLUBILITY CHARACTERISTICS

### 1.2.1. Polyhydroxyanthraquinones

Anthraquinones with only -OH groups in α position dissolve in sodium hydroxide, but are insoluble in alkaline carbonates or bicarbonates, while anthraquinones with the -OH group in β position, with or without an -OH group in α, dissolve both in sodium hydroxide and in carbonates. This difference in solubility is explained by the formation of a chelated ring in the α hydroxyanthraquinones between the quinone =CO group and -OH in α position; as this hydroxyl group is no longer entirely free, it looses some of its activity compared to the -OH in β position.

The special solubility of some anthraquinones rich in β hydroxyls in bicarbonates should be pointed out; such a solubility is usually characteristic of anthraquinones with a -COOH function.

Note that the presence of three hydroxyls on the same ring causes /15
instability of the anthraquinone in alkaline solutions, in which they are easily oxidized with breaking of the anthracene skeleton (decoloration of alkaline solutions). This instability is much greater when the three hydroxyl groups are contiguous.

### 1.2.2. Polyhydroxyanthranoles-anthranones.

They are more or less soluble in sodium hydroxide.

### 1.2.3. Bianthraquinones.

As opposed to anthraquinones, they are always soluble in carbonates, even when they only have α-hydroxyl groups; their solubility in bicarbonates seems to be quite general.

## 1.3 COLORED REACTIONS

Numerous reagents are used.

### 1.3.1. Reagents for polyhydroxyanthraquinones.

#### 1.3.1.1. Magnesium acetate

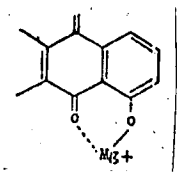
According to Shibata et al., (1950), those polyhydroxyanthraquinones with at least one α-hydroxyl give a characteristic color with a 5% magnesium acetate solution in methanol; this test can be performed either in solution or on paper (need to heat to 90°). This reaction is explained through the formation of a lacquer. The chelated hydrogen can easily be replaced by certain metals, with the formation of a new chelated ring and yielding a complex, highly colored salt. The coloration is purple for compounds with hydroxyls in 1-4, reddish orange if they are in 1-3 and 1-8, and bluish violet if they are in 1-2.

#### 1.3.1.2. Zirconium nitrate.

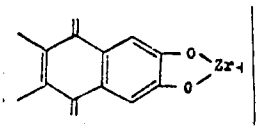
According to Feigl (1960), polyhydroxyanthraquinones with two -OH in ortho positions will give a red to purplish violet coloration with a 4% zirconium nitrate solution in dilute hydrochloric acid. This is explained through the formation of a colored lacquer, in which the zirconium is bound to the two hydroxyls in ortho, according to the schematic below. Feigl has in effect made a general test out of this: in alkaline solutions, hydroxyanthraquinones yield a violet or red precipitate in the presence of a zirconium salt solution. If the anthraquinones do not have two hydroxyls in ortho, the bond is made with the quinone =CO group, as in the case of magnesium acetate, but in acid solutions this bond is unstable and the precipitate is discolored.

The reaction of anthraquinones with two -OH in ortho is immediate /16 on paper, with bright colorations; other anthraquinones react

Magnesium acetate



Zirconium nitrate



slowly and the coloration is dull.

1.3.1.3. Alkaline solutions

In alkaline media, the polyhydroxyanthraquinones yield red to reddish violet solutions (Borntrager reaction). Some unusual exceptions: anthragallol, for instance, takes first a green color, then brown.

1.3.1.4. Sodium hydrosulphite

An alkaline solution of hydroxyanthraquinone is reduced by addition of sodium hydrosulphite. The initially red or violet solution becomes pale yellow; by simple shaking in air it is reoxidized and the original color reappears (general quinone reaction).

1.3.2. Reagents for anthranoles.

1.3.2.1. Mecke's reagent.

It is a mixture of sulfuric acid and selenious anhydride. If it is added to a chloroform solution of anthranol, a bluish-green color appears, that becomes bluish-black rapidly. The original reaction, which we owe to Tunmann, was carried out with anthranol crystals (Schmidt, 1955).

Anthraquinones yield a red color with this reagent.

1.3.2.2. Alkaline solutions

In alkaline solutions anthranols have a yellow coloration, which changes to red in the long run, due to the spontaneous oxidation to anthraquinone.

1.3.3. Reagents of bianthraquinones.

1.3.3.1. Magnesium acetate

Bianthraquinones, just as anthraquinones, react positively with magnesium acetate.

1.3.3.2. Concentrated sulfuric acid.

Some bianthraquinones (those with a -OH group in ortho with the bond) yield an emerald green color, after a transitory purple coloration.

1.4. Spectrophotometry

1.4.1. Polyhydroxyanthraquinones.

Various observations and rules come from the works of Morton and Earlam (1941), Spruit (1949), Briggs et al. (1952), Peter and Sumner (1953), Birkinshaw (1955) and Frank and Reschke (1960); I /17 have supplemented them after studying the absorption curves of hydroxyanthraquinone control samples (M. Gabriel, 1961). The essentials are summarized below.

Polyanthraquinones are characterized by four absorption bands:

210 - 230 mμ,
240 - 265 mμ,
265 - 295 mμ,
400 - 550 mμ.

A typical absorption curve presents a sharp drop in the range of 300 - 400 mμ (Birkinshaw observed a band or inflexion between 310 - 350 mμ for certain anthraquinones with several β-hydroxyls).

The absorption in the 265-290 mμ band is entirely due to the presence of -OH in β position, and is much reduced for -OH in α positions; inversely, the absorption at the 240-265 mμ band is enhanced for -OH in α position, giving rise to several maxima (what Birkinshaw called fine structure).

The introduction of a -COOH group has an effect similar to that of a -OH in β-position, or at least it will have the effect of suppressing the fine structure of the curve in the U.V.

The absorption band in the visible region, 400-550 mμ, is related to the α position of the -OH; a bathochrome effect due to the number of -OH groups was noted (Briggs, 1952).

1.4.2. Anthranoles.

Their absorption in the U.V. is similar to that of the corresponding anthraquinones and only the absorption in the visible region is modified, in the form of a shifting towards the shorter wavelengths (hypsochromic effect) (Awe and Kummel, 1960).

1.4.3. Bianthraquinones

According to Shibata et al.(1956), the spectrum of a bianthraquinone resembles that of the simple anthraquinone that forms it. I have observed, however, looking at the diagrams of that author, that some maxima are at times less marked or even absent in the case of the bianthraquinone.

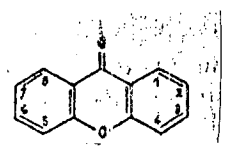
1.5. CHROMATOGRAPHY.

Shibata and Takido (1950 and 1955) have separated numerous natural anthraquinones of Penicillium or of lichens by paper chromatography, using mostly the following two solvents in their analyses:
I. Petroleum ether (45-70°) saturated with 97% methanol. /18
II. Acetone - Petroleum ether (60-70°) - water: 50-50-35.

2. XANTHONES

The reader is referred to J.Roberts' work (1961)

2.1. Formulation.



The most frequent substitute groups are -OH and $-CH_3$. Natural xanthones always have a -OH group in positions 1 or 8.

2.2. COLORED REACTIONS.

The naturally yellow xanthones become intensely yellow in the presence of alkali.

The 1-hydroxy or 8-hydroxy xanthones often yield a green color with ferric chloride.

2.3. SPECTROPHOTOMETRY.

2.3.1. In the ultraviolet.

The spectra of hydroxyxanthones typically present 3 or 4 maxima in the region between 210 and 420 mμ, all of which have strong extinction coefficients.

2.3.2. In the infra-red

A strong band in the frequency region 1660 $cm^{-1}$, due to the carbonyl group, should be noted. For the case of 1-hydroxy or 8-hydroxyxanthones, the frequency is lowered to 1600 $cm^{-1}$ due to chelation with the hydroxyl group.

The other bands, due to various phenyl, methyl, hydroxyl, etc. groups, need no special comments.

## PERSONAL RESEARCH ON BOLETUS

### INTRODUCTION

Boletus are distinguished from other hymenomycetes by the presence of tubes lined with the hymenium on the lower surface of the mushroom's pileus. These tubes are easily separated from the pileal flesh as a consequence of a difference in consistency due to the gelification of the walls of the hyphae; this permits a clearcut differentiation between boletus and polyporus. Under a microscope one also observes a more or less marked bilaterality in the texture of the tubes. Among the gilled types phylloporus, paxillus and gomphidius, one again finds this gelification and this bilaterality in the texture of the lamellae. The similarity between some of these and the boletus is unarguable, as in the case of phylloporus and gomphidius, whose spores are spindleshaped like those of many of the boletus.

Fries (1874) had placed all the pored types in the genus boletus, which was later rejected.

The table below summarizes the most recent classification of the boletus and closely related types (Singer, 1962). I have not taken into account that the genera are limited to French species. I have used Singer's classification in the presentation of my results.

PAXILLACEAE
GOMPHIDIACEAE
BOLETACEAE:
- GYRODONTOIDEAE: Gyroporus, Gyrodon
- SUILLOIDEAE: Boletinus, Suillus (= Ixocomus)
- XEROCOMOIDEAE: Phylloporus, Xerocomus
- BOLETOIDEAE: Pulveroboletus, Boletus (= Tubiporus), Leccinum (= Krombholzia), Tylopilus

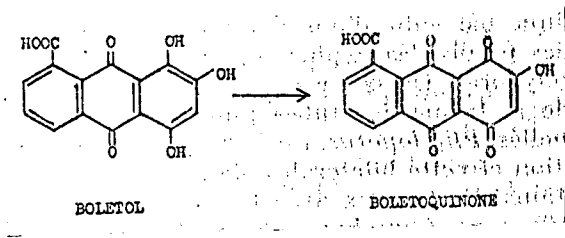
STROMBILOMYCETACEAE: Strobilomyces, Porphyrellus

The bluing of the pulp of Boletaceae was observed only after a long time; this characteristic separates them from the Aphyllaphorae and true Agaricales, and may justify the creation of an independent order of Boletales.

Ever since G. Bertrand's work (1901-1902) it was known that this /20
blue color that some boletus turn at the cut surface is due to the enzymatic oxidation of a yellow pigment, which he called boletol. He recognized that boletol was a phenolic acid capable of being oxidized to boletoquinone of a reddish bluish color due to the presence of alkaline earths and alkali salts. Later Kögl and Deijs (1935) succeeded in obtaining the formulae of boletol and its oxidation product. The latter was obtained by in vitro oxidation with lead tetra-acetate in acetic medium; the boletol is converted to a red p-diquinone, which yields a blue solution when dissolved in a sodium carbonate solution; the color is identical to that obtained in vivo.

It should be pointed out that the authors mentioned above obtained the isoboletol predicted by the formula (see Review of the Chemistry, pp.7-8) at the same time as they obtained boletol in their synthesis. The isoboletol has the same properties and the same spectrum as has boletol (Leger, 1936).

The distribution of boletol among the species is much less well known. G. Bertrand and Kögl were interested mostly in obtaining some boletol and in elucidating the mechanism of oxidation and the formula. G. Bertrand reported that the yellow alcoholic extracts of cyanescens, luridus, satanas, pachypus and lupinus turn blue on addition of laccase; that the same occurred for some nonbluing boleta, chrysenieron, subtomentosus, and that these species do contain boletol. With respect to Kögl, he obtained the boletol from satanas, luridus and badius. Later, Gilbert (1931) affirmed that all species with yellow pulp contained boletol; on




the other hand, to him cyanescens does not contain any. Singer mentions the frequent presence of boletol in his family of Boletoideae and its absence in gyroporus cyanescens.

I shall pay particular attention to completing my knowledge of the presence of boletol in the boletus and related species, without however neglecting other pigments that might be of taxonomic interest.

The species I studied are indicated in Table I; they are taken in Singer's sense and I have observed the synonymy of some genera and species.

EXPERIMENTAL SECTION /21

## 1. STUDY OF THE PIGMENTS

### 1.1. EXTRACTION

To prevent the enzymatic oxidation of boletol and, should such be the case, of other pigments, we rapidly drop the pieces of carpophores into boiling ethanol (G. Bertrand, 1902). For some species the alcoholic extract is not stable; for instance for G.glutinosus the yellow extract from the base of the stem rapidly turns a blackish brown; we resorted to ethyl acetate. For some Suillus we have used acetone since some pigments of this genus rapidly disappeared from alcoholic solutions but lasted longer in acetone.

Extraction and crystallization of a boletol control sample:

We proceded by the method of G. Bertrand (1902) starting with species rich in boletol (B. erythropus, B. luridus) by a lead acetate precipitation from neutral media.

### 1.2. CHROMATOGRAPHIC ANALYSES.

The following two solvents allow for a good separation* :
(a) Isoamylic alcohol - pyridine - water: 30-20-15.
(b) Butanol - acetic acid - water: 40-10-50.

Solvent (a) was the solvent that turned out to be selective for the anthraquinones of the Cortinaria; the Rf of the boletol control being 0.54.

Solvent (b) is Partridge's classical solvent, for which the Rf

* Ascending chromatography, Arches 302 paper

of the boletol control sample was: 0.29.

We have also used a very aqueous solvent, acetic acid - water: 15-85 or 30-70.

1.3. NATURE OF THE PIGMENTS.

1.3.1. Characterization of boletol.

It was very important to be able to perfectly characterize boletol in the various species, be it on chromatograms or in solution using various reagents, before starting research on boletol in these species.

A solution of boletol is yellow-orange; in contact with magnesium acetate the solution becomes citrin yellow and then, several hours later, brown-orange. Zirconium nitrate does not show the reaction /22 expected for the presence of two hydroxyl groups in ortho. The solution takes a transitory blue color with sodium hydroxide that rapidly disappears to make place for an orange color. Sulfuric acid does not cause a color change.

A few drops of potato juice, containing phenoloxydase, change the color of a dilute alcoholic solution to green, then to blue.

Boletol is soluble in bicarbonate solutions.

We observed that in the long run boletol disappears from concentrated, crude alcoholic extracts.

Boletol is easily characterized on chromatograms, where the stain that is yellow in the visible is orange in the U.V. It reacts definitely with potato juice, taking first a green, then a blue color.

The U.V. absorption curve* showed the following maxima:
λM ethanol: 215 - 257.5 - 395 mμ (Figure 1, page 27).

In not having a maximum in the visible region, boletol stands out in relation to other anthraquinones with 2 -OH groups in α.

1.3.2. Characterization of a pseudo boletol.

Chromatographic analysis of the extracts of B. piperatus and of B. parasiticus revealed another yellow pigment apart from boletol, with the same fluorescence, reacting in the same manner with potato juice and with similar Rf values: Rf 0.45 in butanol - acetic acid (boletol 0.29), Rf 0.62 in iso-amylic alcohol - pyridine - water (boletol 0.54) and Rf 0.4 in acetic acid - 30% water (boletol 0.60). We have called it pseudo boletol. We have found it again among the Gomphidea, where it does not accompany boletol. We have isolated it particularly from the base of the stem of G. glutinosus.

The solution of pseudo boletol acts like a boletol solution in the presence of magnesium acetate, sodium hydroxide and potato juice; however, with the sodium hydroxide one obtains the final brown-orange color directly, without passing through the intermediate blue color. Just like boletol, it is soluble in bicarbonates.

Its U.V. absorption curve can be superimposed on that of boletol (Figure 1, page 27):
λM ethanol: 215 - 255 - 390-95 mμ.

We observed that in the extracts the pseudo boletol seemed to be more stable than boletol. /23

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* For the U.V. spectrophotometry we used the apparatus of Jobin and Yvon.

1.3.3. Characterization of other pigments.

We isolated some yellow pigments different from boletol from several Suillus. Chromatographic analyses in three different solvents -- (a), (b) and acetic acid - water (15-85) -- revealed the existence of a pigment common to flavidus and luteus, and another one common to elegans and tridentinus.

The action of various reagents points to anthraquinone type pigments.

They are soluble in bicarbonate solutions.

The U.V. absorption curves show the following maxima:

| | | |
|---|---|---|
| luteus | λM ethanol | 218 - 275 - 385 mμ |
| flavidus | λM ethanol | 218 - 280 - 380 mμ |
| elegans | λM ethanol | 218 - 277.5 330-390 mμ |
| tridentinus | λM ethanol | 218 - 275 - 320-390 mμ |

Yellow pigment from the foot of piperatus:

We do not know the nature of this pigment, but we can say with certainty that it is not an anthraquinone type pigment. The pigment, although very soluble in water, is not a glycoside.

On its absorption curve we observed (Figure 2, page 28 ):

λM water: 320 - 395 mμ

Some other pigments, sometimes quantitatively more important than boletol, could be isolated from boletus and deserve further study: a yellow pigment in Phylloporus rhodoxantus, a yellow-brown pigment in Paxillus involutus and Gyrodon lividus, a pigment that turns pink in ethanol, from bovinus and Gomphidius helveticus

TABLE I. BOLETUS AND RELATED SPECIES. DISTRIBUTION OF BOLETOL AND PSEUDOBOLETOL.

| Genera | Species | Bluing in air | Boletol | Pseudoboletol |
|---|---|---|---|---|
| BOLETUS Dill. ex Fr. (=TUBIPORUS Karst.) | edulis | | * | |
| | reticulatus | | * | |
| | pinicola | | * | |
| | calopus | bl. | * | |
| | appendiculatus | bl. | * | |
| | pulverulentus | bl. | * | |
| | luridus | bl. | * | |
| | erythropus | bl. | * | traces |
| | rhodoxanthus (purpureus) | bl | * | |
| TYLOPILUS Karst. | felleus | | | |
| LECCINUM S.F.Gray (= KROMBHOLZIA Karst.) | nigrescens /(crocipodius) | | | |
| | aurantiacum | | | |
| | carpini | | | |
| | scabrum (leucophaeus) | | | |
| PHYLLOPORUS Quel. | pelletieri (rhodoxanthus) | | * | |

| Genera | Species | Bluing in air | Boletol | Pseudoboletol |
|---|---|---|---|---|
| XEROCOMUS Quel. | chrysenteron | bl. | * | |
| | subtomentosus | | * | |
| | parasiticus | | traces | * |
| | badius | bl. | * | |
| BOLETINUS Kalchbr. | cavipes | | | |
| SUILLUS Micheli ex S.F. Gray (= IXOCOMUS Quel | aeruginascens (viscidus) | bl. | * | |
| | tridentinus | | | |
| | grevillei (elegans) | | | |
| | flavidus | | | |
| | luteus | | traces | |
| | granulatus | | | |
| | placidus | | * | traces |
| | bovinus | bl. | * | |
| | variegatus | bl. | * | |
| | piperatus | | * | * |
| GYRODON Opat. | lividus | bl. | * | |

/25

| Genera | Species | Bluing in air | Boletol | Pseudoboletol |
|---|---|---|---|---|
| GYROPORUS Quel. | castaneus<br>cyanescens | <br>bl. | | |
| STROBILOMYCES Berk. | floccopus (strobilaceus) | | | |
| PORPHYRELLUS Gil. | pseudo-scaber (prophyrosporus) | | | |
| GOMPHIDIUS Fr. | helveticus<br>viscidus<br>glutinosus | | | *<br>*<br>* |
| PAXILLUS Fr. | atrotomentosus<br>panuoides<br>involutus | | | |

and viscidus, etc...

## 2. DISTRIBUTION OF THE PIGMENTS.

We only summarize the distribution of boletol and pseudoboletol (Table I.)

### Taxonomic interest in the study of pigments

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Boletoideae: This is the most typical Boletus, characterized by long tubes, independent of the stem, fine pores and, microscopically, by long, spindle-form spores.

The two genera Boletus and Leccinum make up almost all of the subfamily. All the Boletus examined contained boletol; in edulis, a species of white pulp, the boletol is easily discernible in the yellow tubes of old individua. On the other hand, some of the Leccinum contain it, like crocipodius in the yellow tubes and aurantiacum at the base of the foot, often speckled with blue.

The majority of the species of these two clearly identified genera (the most evolved among the Boletus) react with phenyloxidase, but in different ways: the former by bluing (caused by the presence of boletol) the latter by blackening.

With respect to Tylopilus felleus, grouped by Singer among the Boletoideae, it does not contain boletol, nor does it blacken.

Xerocomoideae: all species contain boletol; parasiticus nevertheless contains only traces and is unusual due to the presence of pseudoboletol.

Phylloporus rhodoxanthus contains as much boletol as the other species.

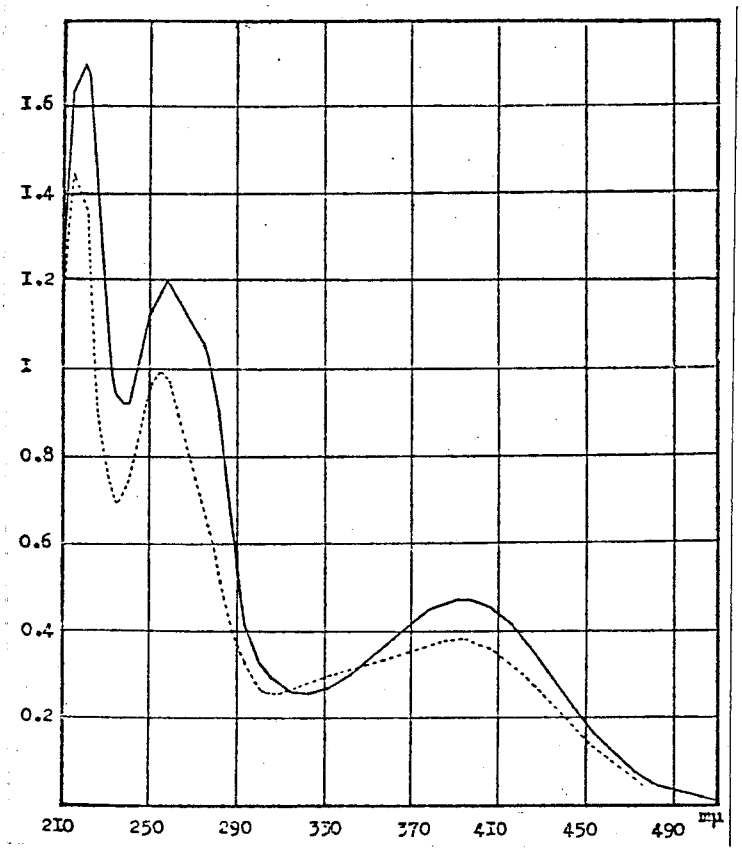


Figure 1. Absorption curves of boletol and pseudoboletol (95°ethanol). Continuous line: boletol. Dotted line: pseudoboletol.

Suilloideae: we found boletol in Boletinus cavipes.

We established that Suilla, which however show a great homogeneity in their morphological characteristics, do not all contain boletol; viscidus is a polymorphic species, and some of its forms have a completely white pulp which should not contain any boletol (R. Kühner, 1927); in piperatus the boletol is located at the level of the tubes, which also contain pseudoboletol.

Gyrodontoideae: we have not found boletol in the Gyropora, even

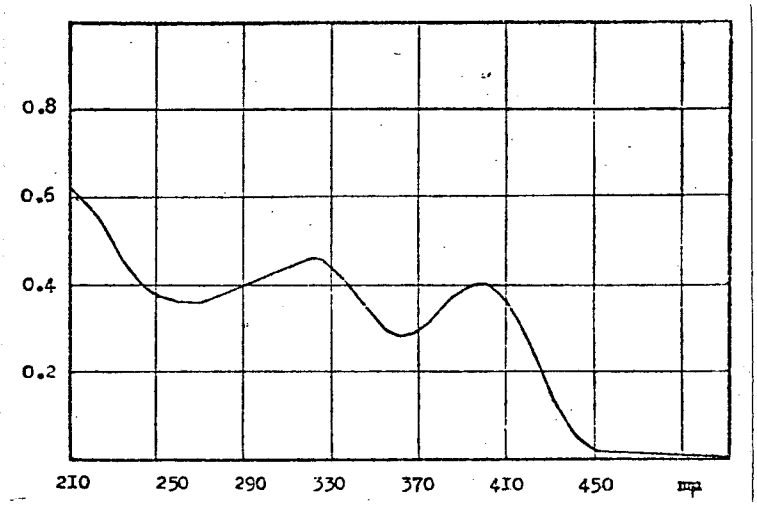


Figure 2. Absorption curve for the yellow pigment from the foot of B. piperatus (distilled water).

in cyanescens, while Gyrodon lividus indubitably contains it.

Boletol, widespread throughout the Boletaceae, is missing in the two strobilomycetaceae studied.

Among the gilled species close to the Boletaceae, only Phylloporus rhodoxanthus has boletol; it should be pointed out that mycologists consider this species inseparable from Xerocomus subtomentosus.

On the other hand we have not found boletol among the Gomphidiaceae and the Paxillaceae. One must however point up the fact that in the family of Gomphidiaceae, notably close to Boletaceae in their spindle shaped spores, we have always found pseudoboletol.

The Paxillaceae, farthest removed from Boletus due to their non-spindle-shaped spores, contain neither boletol nor pseudoboletol.

## PERSONAL RESEARCH ON CORTINARIA

### INTRODUCTION

The genus Cortinarius Fr. was divided by Fries into several sub-genera: Myxacium, covered completely with a sticky pellicle (their foot is hence sticky as is their pileus; Phlegmacium, in which only the pileus is sticky (the Cortinaria with marginate bulb: Scauri, belong to this sub-genus); Hydrocybe and Telamonia, which are not sticky, but have a hygrophane pileus, and finally, Dermocybe and Inoloma, which are not sticky and the pileus of which is not hygrophane.

In a number of these sub-genera one can observe great differences in color, according to the species. R. Kühner (1949) showed that it is important to consider the microscopic localization of the pigments. Just as most of the ochrophore Agaricales, the Cortinaria have in their carpophores hyphae of membranelike pigments, just as their spores have them. They often form colored platelets or more or less fine incrustations on the outer surface of cell walls; they are usually brown or yellow, often situated on the surface of the pileus, although they may appear in the hyphae deep inside the pulp, the stroma, the gills or the foot, in the numerous hygrophane species. These pigments are difficult to extract.

On the other hand, the blue and violet pigments that diffusely color the cytoplasm are very characteristic of the genus Cortinarius; one finds them throughout the sub-genera, although one does not find them elsewhere except very rarely. These pigments seem to change very fast since the coloration they impart to the carpophores are not often found outside of the yellow parts (pileic rim, top of the foot, yellow platelets). Finally, some other pigments are intercellular or vacuolar, of different colors such as olive, yellow or red, for the most part; these two types of pig-

ments are easily extracted and relatively stable. These are the only ones we have undertaken to study until now. This excludes from our work all the Myxacium and most of the Hygrophana, of which we only studied bulliardi because of the red color at the base of the foot.

## SUB-GENUS DERMOCYBE Fr. SENSU ORTON 1958 /30

### Botanical Introduction

### LIMITS OF THE SUB-GENUS

The sub-genus was reduced by P.-D. Orton and R. Singer to E.Fries' section *** (species with red and yellow platelets); it corresponds to a combination of R. Kühner and H. Romagnesi's sections Sanguinei and Cinnamomei, sections distinguished by means of the color of their platelets: red in Sanguinei and orange-yellow or olive in Cinnamomei.

### SPECIES AND VARIETIES STUDIED.

SANGUINEI:

sanguineus (Wulf. ex Fr.) Fr., semisanguineus (Fr.) Gillet, phoeniceus (Bull.) Maire, cinnabarinus (Fr.).

CINNAMOMEI:

uliginosus Berk., sphagneti Orton (= cinnamomeus variety paludosus Fr.), olivaceofuscus Kühner, cinnamomeolutescens Henry (=cinnamomeus sensu Ricken, Lange), conformis Fr., croceus Fr., malicorius Fr.

Henry described a number of Cinnamomei species (1939). Several of them were taken up again by P.-D. Orton (1958), to whom we owe

some very clear synoptic tables; these gave me the first general insight into a section in which the specifications seemed far from being perfect.

The first three species on our list are taken in the sense of R. Kühner and, it seems, Orton. My cinnamomeolutescens seems to be Orton's, notably for their relatively large spores, (8.2) - 9 - 10 - (11) x 5.2 - 6 - (6.5) µ. On this species we made two collections in the Haut-Beaujolais (1959-1960) and one collection in the Echets (K-Ly. 61-55.); we have also studied one collection from the Haut-Beaujolais (K-Ly. 61-41.) that conformed well to Lange's (Lange, 1935-1940) image, who however indicated smaller spores, 7.7 - 8,2 x 4.5 - 5µ.

We take malicorius here in the sense of R. Kühner (1960), characterized, in particular by the absence of yellow extracellular masses in the stroma of the platelets and by their especially small spores, 5.5 - 5.7 (6) x 3.7 - 4 - 4.2µ, on the carpophores from which we extracted the pigments; this is not Orton's malicorius, which shows pigmentary masses in the platelets and larger spores (5.5) - 6 - 8 x 4 - 5µ; for those two characteristics, Orton's mushroom is much more like our conformis, which gave us /31
spores of 6.5 - 7 - 7.5 - (8.5) x 4 - 5µ; we believe they belong to the same species.

We gave the name croceus to one collection from the Haut-Beaujolais (K.Ly. 61-38); the mushroom corresponded rather well to a plate by Lange (plate No. 95, Lange, 1935-1940), but less well to the description given; this mushroom is certainly very close to conformis, both for the color of its platelets and the spore dimensions, 7.7 - 8 x 4.5 - 4.7µ, but we believe they are different, in opposition to what Orton thought when he claimed synonymy for conformis and croceus with croceofolius Peck; elsewhere his croceofolius has much smaller spores.

## EXPERIMENTAL SECTION

### 1. STUDY OF THE PIGMENTS.

#### 1.1. EXTRACTION.

The short time of appearance of the carpophores inevitably led to a need for stocking. One can plan either on stocking the dried carpophores, or on stocking the extracts of fresh material. We usually proceeded with the extraction of fresh material, to prevent oxidation of the pigments (anthranols, for instance).

Boiling ethanol is used as a solvent, into which the bits of carpophores are dropped; boiling is maintained for a quarter of an hour at least, to completely stop any enzymatic action. Sometimes we have resorted to chloroform to extract the extracellular pigments selectively.

As an exception we used dry carpophores for two species, olivaceofuscus and sphagneti, since we only had dried species at our disposal. The dried samples were not more then a few weeks old (of the comparative extractions we carried out for some species, between fresh and dried carpophores, we never found significantly different results).

We never performed a total extraction. It is quite likely that some pigments are not extracted at all, notably as I have had a chance to verify (M. Gabriel, 1960 b),the brownish pigments responsible for the dark coloration of some species, corresponding to incrustations in the membrane.

#### 1.2. CHROMATOGRAPHIC ANALYSIS.

After some tests with Shibata's solvents I and II* (for their composition see page 14) and others, such as butanol - acetic acid -

*See the composition of these solvents p.14

TABLE II

SUB-GENUS DERMOCYBE. PIGMENT LIST

(Chromatography in isoamylic alcohol-pyridine-water)

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| | Rf | Aspect and colored reactions | | | |
|---|---|---|---|---|---|
| | | visible | U.V. | ammonia | magnesium acetate |
| 1 | 0.90 | yellow-orange | lemon-yellow | pink | pink |
| 2 | 0.86 | light purple | non fluorescent | light purple | light purple |
| 2' | 0.82 | yellow | orange | yellow | yellow |
| 3 | 0.80 | yellow | orange | pink | pink-orange |
| 3' | 0.60 | pale yellow | non fluorescent | blue-gray | light purple- gray |
| 4 | 0.50 | light purple-purple | non fluorescent | light purple-violet | light purple-violet |
| 5 | 0.39 | pink | light pink | light purple | purple |
| 6 | 0.34 | yellow | dull orange | pink | orange |
| 6' | 0.30 | pink | flesh | light purple | violet-blue |
| 7 | 0.28 | orange-yellow | orange yellow | rose-light purple | purple |
| 8 | 0.18 | pink | light purple | violet | light purple-violet |
| 9 | 0.14 | yellow | orange-brown* | transient orange | orange-yellow |
| 10 | 0.13 | orange-pink | orange | purple | violet |
| 11 | 0.05 | light purple | little fluoresc. | light purple | light purple |
| 12 | 0.04 | red | purple | red | red |
| 13 | 0 | very pale yellow | luminous bright ylw. | yellow | non |

* Color partially masked by blues

water (40-10-50) and water - acetone (30 - 2.5), I found that the solvent iso-amylic alcohol - pyridine - water (30-20-15) was the most interesting. Separation takes place by ascending chromatography on Arches 302 paper, in cylindrical tanks (9.5 x 48 cm). /33

Depending on the species, 6 to 10 pigments are thus shown, with Rf values ranging from 0.04 to 0.9 (Figure 3). They are yellow, orange, pink or light purple. Almost all colors turn pink or violet in ammonia vapors and take a characteristic coloration after spraying with magnesium acetate. Most of them show a fluorescence under "Wood's light."

The list of pigments and the observations made on them are given in Table II. The pigments are numbered in order of decreasing Rf values.

One could fear that the pyridine used in the solvent might cause a supplementary spot to be formed for the same pigment, due to an ionized or complex form with the pyridine, but chromatograms obtained with other solvents show this not to be the case.

1.3. LIST OF PIGMENTS.

(See Table II.)

The pigments found in cinnabrinus are all of them different from those characterized in the table. We thought this species not to be a Dermocybe; we treat it as farther removed, together with bulliardi.

1.4. STRUCTURE.

1.4.1. Contribution of chromatography.

Colored reactions and structure:

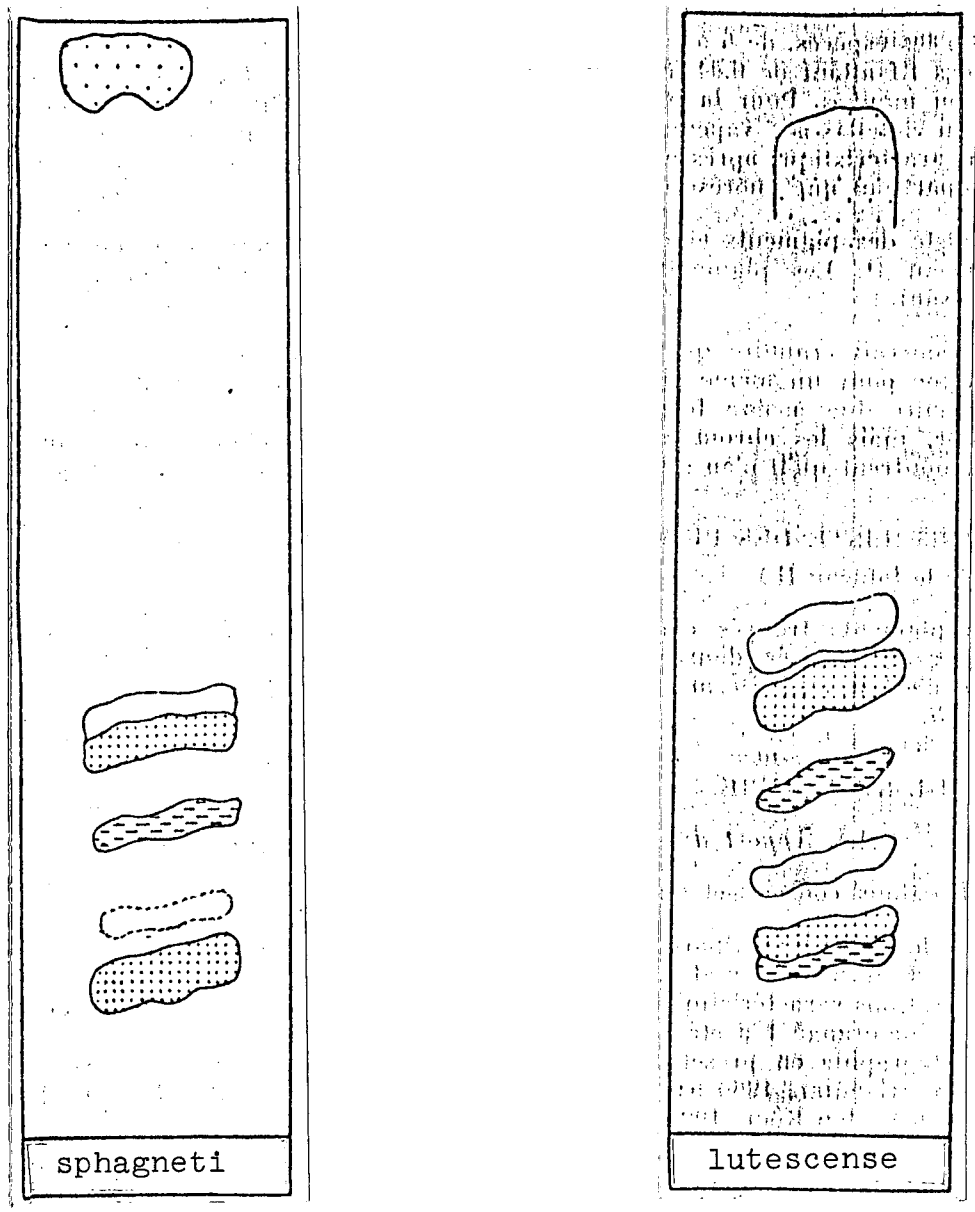





Figure 3. Ascending chromatography on alcoholic extracts of Dermocybe in the solvent isoamylic alcohol-pyridine-water (30-20-15). Dotted:yellow pigments; dashes: orange pigments; blank: pink pigments.

All of the pigments separated except 13 and 2' showed Borntrager's reaction and, after action by magnesium acetate, the characteristic hydroxyanthraquinone colorations. The orange-yellow pigment 1 has been identified as being emodine, after chromatography in the

presence of an emodine control sample obtained from rhubarb (Gabriel 1960 a). The light purple pigment 2 is dermocybine (isolated according to Kögl, 1925 a).

Most of these pigments are stable, they maintain their initial color on the chromatogram, while the bright yellow pigment 2', which does not react with either magnesium acetate or ammonia vapors, becomes dull after a few days and then yields a brownish-orange color with ammonia vapors. We are here dealing with an anthranol, as we shall see later.

With respect to the pale yellow pigment 13, very fluorescent, it is not of an anthraquinone nature.

Rf and structure:

Shibata's solvents do not lead to migration for the Dermocybe pigments, and only one stain is separated in the case of sanguineus and malicorius, which corresponds to emodine.

Considering the Rf values given by Shibata for different anthraquinones, we observed that in solvent I the tri-hydroxyanthraquinones migrated very little less than the methylated ones; the tetrahydroxyanthraquinones were not displaced at all.

Moreover, the α or β position of a hydroxyl group considerably affected the Rf: a single hydroxyl group in β slows the displacement down, and the same is true for the substitutes $-CH_2OH$, and -COOH:

4,5 dihydroxy 2-methyl anthraquinone Rf 0.92
4,5 dihydroxy 2-ethyl anthraquinone Rf 0.15
4,5 dihydroxy 2-carboxyl anthraquinone Rf 0

In Shibata's solvent II (the more polar solvent) we performed the chromatography of control anthraquinones. As in the preceding

solvent, a hydroxyl in β position or the presence of a -COOH group slow the migration down (for instance, for boletol, Rf 0).

We also performed the chromatography of anthraquinone controls in our separation solvent (30-20-15); it was not selective any longer: all the Rf values were between 0.7 and 0.9, whether the anthraquinones had one substitute $-CH_2OH$ or many, or a -OH in β position; however, the presence of a -COOH ssemed to reduce the Rf:

* purpurine Rf 0.9
  boletol Rf 0.5

In conclusion, the hypothesis of anthraquinones having a -COOH substitute is then plausible. One can also think that one is dealing with complex anthraquinones of high molecular weight, for instance, bianthraquinones.

1.4.2. Contributions of solubility characteristics and chemical reactions.

With the aim at defining the structure of the pigments separated by chromatography, we proceded with the isolation of some of them. To begin with, the extraction of certain parts of carpophores /36
rich in a certain pigment (see 2.2 Distribution among different part of the carpophore), then chromatography and elution of the stains:

Yellow pigment 3: platelets from malicorius or conformis.
Light purple pigment 4: platelets of phoeniceus.
Pink pigment 5: pileic cuticle of uliginosus or platelets of conformis
Yellow pigment 6: foot and pileic cuticle of semisanguineus

* Boletol differs from purpurine only in the presence of an additional -COOH.

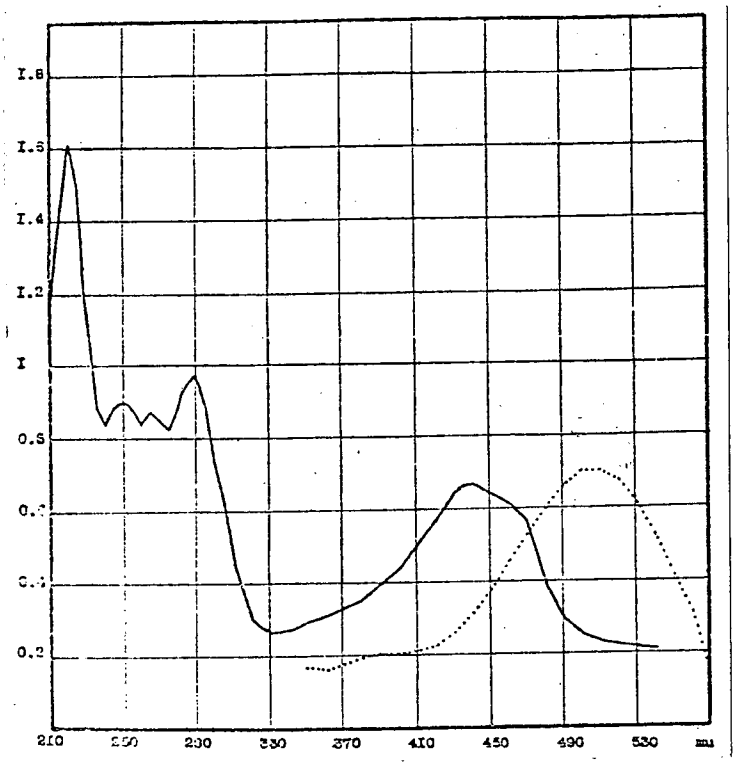



Figure 4. Absorption curve for pigment 1, emodine (95°ethanol). Dotted: in the presence of Mg acetate.

Yellow pigment 2': cinnamomeolutescens or conformis (chloroform extract)

The elution of each pigment from the chromatographic bands yields a pure product, which we have used occasionally to obtain crystals (pigment 3 and 4).

1.4.2.1. Anthraquinones.

The solubility of pigments 3, 4, 5 and 6 in bicarbonate implies either an acid function or a large number of -OH in β position.

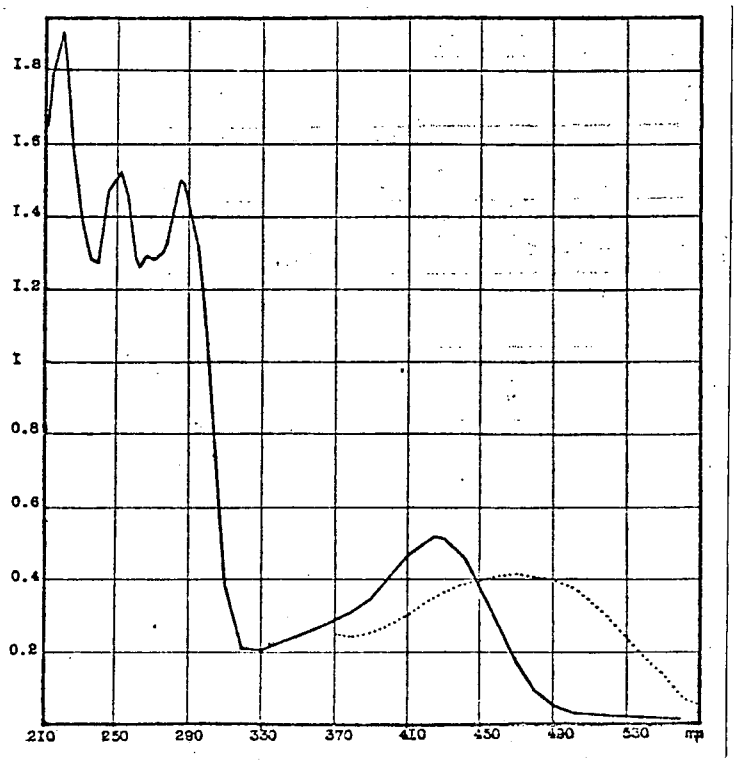



Figure 5. Absorption curve for pigment 3 (95°ethanol). Dotted: in the presence of Mg acetate.

The reaction with zirconium nitrate is positive for the pink pigment 5, which has two hydroxyl groups in ortho.

Bianthraquinones can be split with hot sodium hydrosulphite (Shibata-Murakami, 1955 and 1956). We used this technique on pigments 1, 3, 5 and 6 and only pigments 5 and 6 changed: while always of an anthraquinone nature, they now had a much higher Rf. We observed that this treatment can cause the loss of a hydroxyl in α in the case of some anthraquinones.

1.4.2.2. Anthranol

The yellow pigment 2', which on chromatograms stands out as we

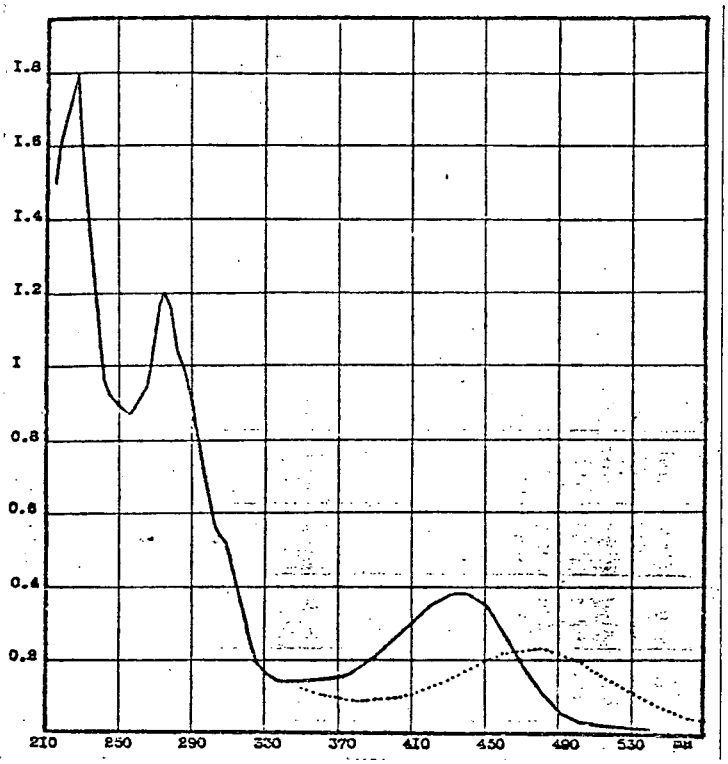



Figure 6. Absorption curve for pigment 6 (95°ethanol). Dotted: with Mg acetate

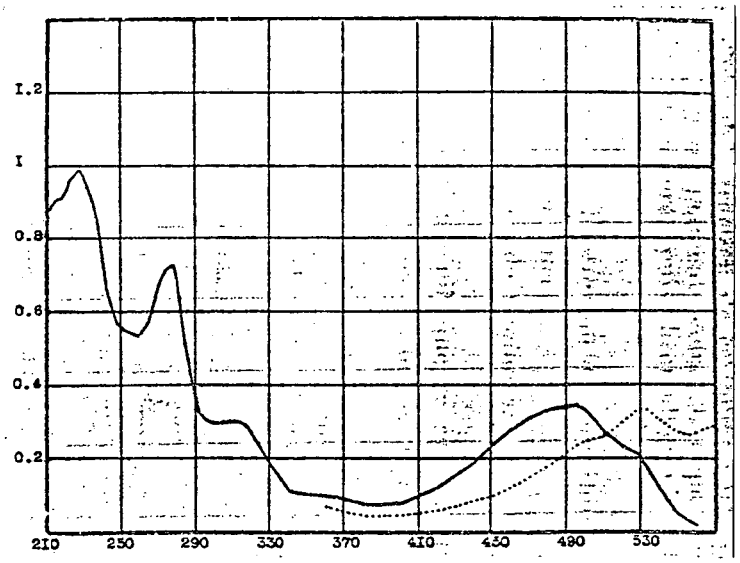



Figure 7. Absorption curve for pigment 5 (95°ethanol). Dotted: with Mg acetate

have already mentioned, can be easily separated by a simple extraction with chloroform in cold; the other pigments are not extracted by this solvent. It passes into an alkaline solution and retains its yellow color. It shows a clear anthranol reaction with Mecke's reagent.

It can be oxidized to anthraquinone by the action of 2% hydrogen peroxide in alkaline medium, heating four to five minutes on a boiling water bath.

The anthraquinone obtained does not correspond to any of the pigments extracted with Dermocybe.

1.4.3. Contribution of spectrophotometry /39

1.4.3.1. Anthraquinones

An examination of the spectra allows the following observations: Emodine characterized and separated by chromatography yields a spectrum identical to that indicated by Birkinshaw (1955), whether it comes from sanguineus or malicorius:

λM ethanol: 222 - 252 - 265 - 289 - 437 mμ,

the spectrum of pigment 3 is similar:

λM ethanol: 220 - 252 - 266 - 285 - 425 mμ.

Both of these pigments have a "fine structure" in their U.V. absorption curve (Figures 4 and 5), while the other pigments studied 4,5,6, and 9 stand out for the simplicity of their curves (Figure 6 and 7).

4 λM ethanol: 215 - 280 ~ 350 - 488 ~ 520 ~ 560
5 λM ethanol: 228 - 278 - 310 - 495 ~ 530
6 λM ethanol: 228 - 275 ~ 308 - 435
9 λM ethanol: 225 - 272.5 ~ 305 - 425

The absence of the 240 - 265 mμ band suggests a predominance of the -OH in β or the existence of a -COOH substitute.

1.4.3.2. Anthranol.

The U.V. spectrum of anthranol brings to mind that of pigments 5 and 6. Its oxidation does not cause any modification in the U.V. region; in the visible a 25 mμ bathochrome shift occurs.(Gabriel, 1961).

The observations made in the preceding paragraphs are in agreement with the hypothesis of a -COOH substitution on the anthraquinone nucleus, without however excluding the possibility of a bianthraquinone structure.

2. DISTRIBUTION OF THE PIGMENTS.

In this section the distribution will be looked upon from a macroscopic point of view; microscopic observations are dealt with later (see microtopography).

The results are summarized in Tables III and IV. The greater or lesser abundance of the pigment is indicated by *.

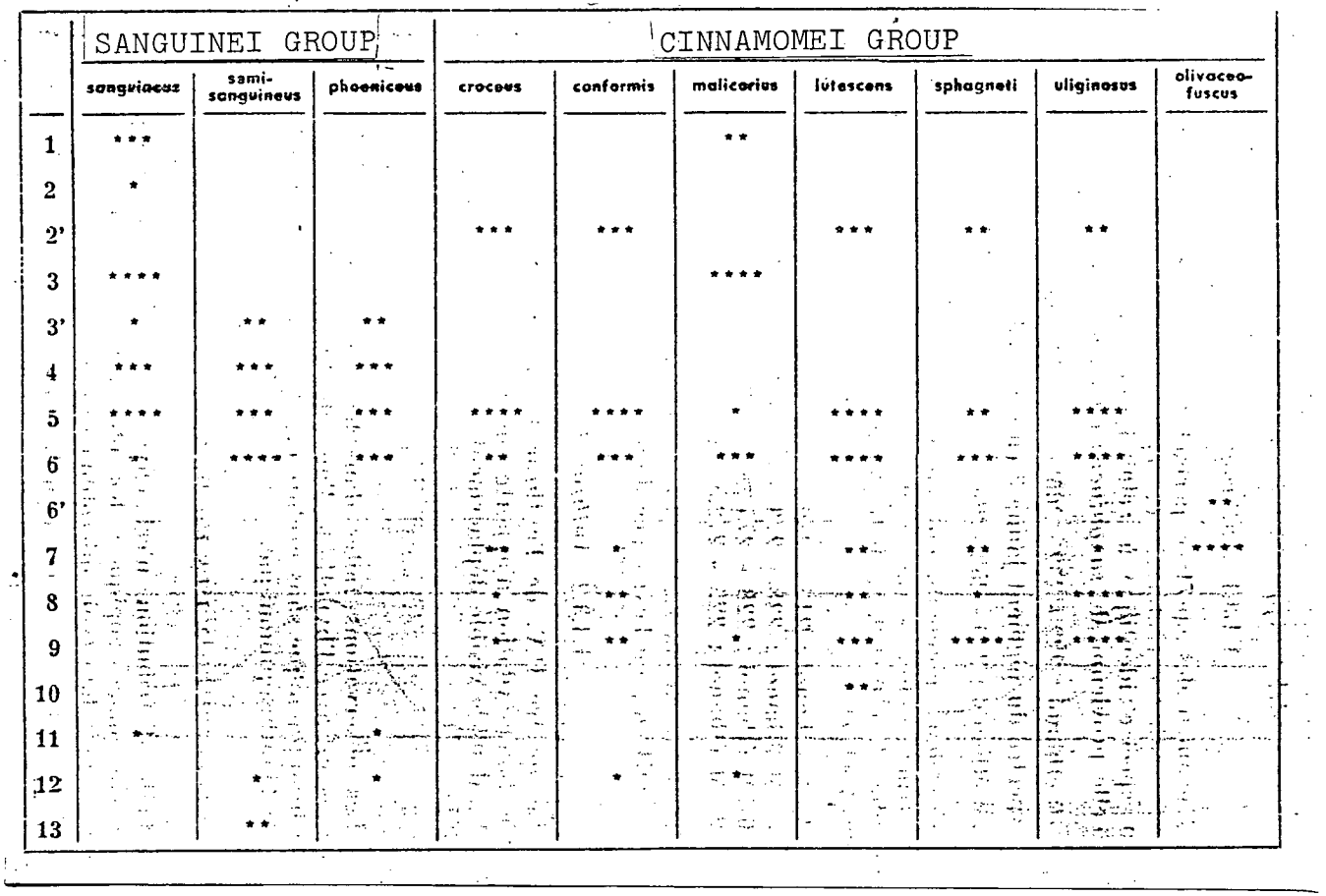
2.1. Distribution according to species (Table III)

2.2. Distribution in the different parts of the carpophore (Table IV)

Scanning these Tables, a mycologist who specializes in Cortinaria /42
might be led to make observations that will astonish him, next to others that are easily explained. Thus, the presence of pink pigments in our extracts of pileic cuticle from phoeniceus or uliginosus does not surprise, since this covering is overtly reddish and under the microscope shows hyphae with a pink content; in a similar manner the covering of the pileus of croceus was found by R. Kühner to have hyphae containing red material.

TABLE III

SUB-GENUS DERMOCYBE. PIGMENT DISTRIBUTION.



| | SANGUINEI GROUP | | | CINNAMOMEI GROUP | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *sanguineus* | *sami-sanguineus* | *phoeniceus* | *croceus* | *conformis* | *malicorius* | *lutescens* | *sphagneti* | *uliginosus* | *olivaceo-fuscus* |
| 1 | *** | | | | | ** | | | | |
| 2 | * | | | | | | | | | |
| 2' | | | | *** | *** | | *** | ** | ** | |
| 3 | **** | | | | | **** | | | | |
| 3' | * | ** | ** | | | | | | | |
| 4 | *** | *** | *** | | | | | | | |
| 5 | **** | *** | *** | **** | **** | * | **** | ** | **** | |
| 6 | ** | **** | *** | ** | *** | *** | **** | *** | **** | ** |
| 6' | | | | | | | | | | |
| 7 | | | | ** | * | | ** | ** | * | **** |
| 8 | | | | * | ** | | ** | * | **** | |
| 9 | | | | * | ** | * | *** | **** | **** | |
| 10 | | | | | | | ** | | | |
| 11 | ** | | * | | | | | | | |
| 12 | | * | * | | * | * | | | | |
| 13 | | ** | | | | | | | | |

TABLE IV

DISTRIBUTION OF PIGMENTS IN DIFFERENT PARTS OF THE CARPOPHORE

| | PHOENICEUS | | SEMISANGUINEUS | | CONFORMIS | | | LUTESCENS | | | ULIGINOSUS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FOOT yellow, red stripes | PLATELETS blood red | CUTICLE reddish ocre | FOOT yellow | PLATELETS blood red | CUTICLE yellow brown | FOOT yellow | PLATELETS orange | CUTICLE fawn | FOOT brown-yellow | PLATELETS yellow, brown, olive | CUTICLE yellow, brown | FOOT red | PLATELETS yellow | CUTICLE red |
| 1 | | | | | | | | | | | | | | | |
| 2 | | | | | | | ** | ** | *** | | ** | | ** | * | |
| 2' | | | | | | | | | | | | | | | |
| 3 | | ** | *** | | | ** | | | | | | | | | |
| 4 | | **** | | | **** | | | | | | | | | | |
| 5 | **** | **** | **** | ** (1) | **** | **** | **** | **** | **** | **** | * | * | **** | ** | **** |
| 6 | **** | **** | ** | **** | **** | **** | **** | *** | *** | **** | **** | * | ** | **** | |
| 6' | | | | | | | | | | | ** | | | | |
| 7 | | | | | | | | ** | * | | **** | * | | | |
| 8 | | | | | | | | ** | * | | * | ** | *** | * | **** |
| 9 | | | | | | | ** | ** | *** | | **** | ** | *** | *** | * |
| 10 | | | | | | | | | | * | | ** | | | |
| 11 | ** | * | | | | | | | | | | | | | |
| 12 | * | ** | ** | ** | | ** | * | | | | | | | | |
| 13 | | | | *** | | | | | | | | | | | |

(1) Localized primarily at the base of the foot.

It is not unusual to find an abundance of pink pigments 5 and 8 in the platelets of conformis, next to the yellow pigments 2', 6 and 9, and orange pigment 7, because their mixture can account for the saffron-like or marigold coloration of the platelets of this mushroom. The comparison of the chromatograms of platelet extracts of conformis and cinnamomeolutescens shows much clearer than any table could, that the pinks are less concentrated in the latter, which could explain the more citrine-yellow color (sub-olive) of its platelets. It is the same for a collection of cinnamomeolutescens (Ly.61 K 55 - K 41)where the extract of the whole mushroom practically shows only yellows.

In the same manner the presence of a purplish pigment in the sanguinei, and notably in their platelets, can be expected, since that is their color; on the other hand, the abundance of yellow pigments in sanguineus is astonishing, since they do not affect its color towards reds plus orange.

It is even more astonishing that the foot of cinnamomeolutescens, which is citrine or sulfur yellow inside and out, is not limited to yellow pigments but may contain, sometimes even in significant quantities, the pink pigment 5.

This leads one to ask oneself if all the pigments that are extracted and separated really exist in the living mushroom.

## 2.3. VALIDITY OF THE RESULTS.

Could some of the pink pigments on the chromatograms come from some modification in the yellow pigments? We know with what ease yellow pigments turn definitely into pink. Now, it is certain that the pink pigments 5 and 8 should be very close in their chemical composition to the yellow pigments 6 and 9, respectively: they are coupled to them on chromatograms and, above all, uliginosus differs from its mutant luteus only in that the pileic covering

has pigments 5 and 8 instead of 6 and 9 (M. Gabriel and D. Lamoure 1965).

Against the hypothesis of a modification of the yellow pigments we can say that the variety luteus of uliginosus never has yellow pigments other than 6 and 9 in the pileic covering; that the /43
pileic covering of sanguineus is rich in yellow 6 but not in pink 5; that the foot and the pulp of conformis have the yellows 6 and 9 without the pinks 5 and 8.

We also observed that, if the majority of the pigments appeared to be clearly fluorescent on the chromatograms, while the carpophores are very little fluorescent, or not at all (with the exception of the foot of semisanguineus) this can be explained by differences in their physico-chemical state.

## Microtopography of the pigmentation



The information related to the microscopic localization of the coloration is owed to R. Kühner (1949, 1955, 1960). This has allowed us to establish a correspondence between the pigments isolated by chemical techniques and the colorations that can be observed in situ by the mycologist.

As the author mentioned shows, pigments that are easily extractible can be divided into two categories: extracellular pigments which accumulate in dead cells (most commonly old basidia) and vacuolar pigments, hence situated inside the living cells.

EXTRACELLULAR PIGMENTS.

In many Cinnamomei R. Kühner observed the presence of bright yellow masses of irregular form among the hypha of the platelets' stroma; they are refractive but not birefractive. They were again found among the hypha of the pulp in both pileus and foot. These yellow masses are not present among Sanguinei; therefore they are not absolutely characteristic of the Cinnamomei as R. Kühner thought originally; he recognized, in fact, that two of the Cinnamomei, malicorius and olivaceofuscus, do not have them. The knowledge of these facts was valuable in knowing to which stain on the chromatogram the interhyphic pigment of common Cinnamomei corresponded. It could not be anything but the yellow pigment 2', that is, anthranol. We were able to verify this with Mecke's reagent on previously dried samples: the interhyphic masses became stained a black blue (verified on cinnamomeolutescens, conformis and uliginosus).

R. Kühner observed that these yellow masses dissolved in ammonia, and that dried specimens turned brownish and reddish in the presence of this reagent; this had to do with the oxidation of anthranol to anthraquinone, similar to the one we had performed in

vitro with hydrogen peroxide.

On some old sphagneti carpophores R. Kühner and D. Lamoure (1960) observed that the big yellow bodies were replaced by an olive-brown mass that could be quite diffused and reminded one of that found in olivaceofuscus (R. Kühner, 1955); now according to our chromatograms, sphagneti does not always have the pigment 2' and in such a case we find, very close to the solvent front, another light brown-yellowish pigment with a cream-colored fluorescence, which resembles a pigment with similar Rf revealed in olivaceofuscus.

VACUOLAR PIGMENTS /45

It is likely that all extractible pigments other than the anthranols are vacuolar pigments. Microscopic observations tend to prove this, but until now we have not succeeded in lozalizing them individually with certainty.

The covering of semisanguineus, in which the vacuola are yellow colored, shows principally the yellow pigment 6 after chromatography. The covering of phoeniceus has both yellow and pink vacuolae; the pink coloration would be due to pigment 5, the only abundant pink on the chromatograms; with respect to the yellow vacuolae, their color would be due to pigment 6 or pigment 3', or to both of them simultaneously. The pink coloration of the vacuolae of the covering in uliginosus is very likely due to the pigments 5 and 8 found in the chromatograms; as for the luteus variety of uliginosus, in the case of the yellow vacuolae one is likely dealing with yellow pigments 6 and 9.

In the pileic covering of sphagneti, R. Kühner reported the presence of orange-yellow vacuolae, in which no ammonia-sensitive change takes place; one is led to believe that the coloration is due to the yellow pigment 9, which is abundant on the chromato-

grams and does not turn red when acted upon by ammonia.

We call attention to still other observations, that show the presence of vacuolar pigments and complete these comments: yellow vacuolar color - that turns red with ammonia - of the hyphae on the pileic surface of malicorius and olivaceofuscus; red vacuolar coloration of the platelet stroma in semisanguineus and phoeniceus (particularly evident on both sides of the median plane, especially towards the hymenopode); uniformly citrine intracellular coloration of the platelet stroma of malicorius, orange coloration of the underside of its hymenium, or that of cinnamomeolutescens.

Taxonomic interest of pigment studies

From their distribution among the various species, pigments can be classified in three categories:

1) Pigments that are peculiar to one or two species, and that differ from other species in the sub-genus or section.

2) Pigments characteristic of a section, in the sense that they are present in the majority if not the totality of the species of a section, but absent in the species of another section.

3) Pigments characteristic of a sub-genus, that is, common for instance to all Dermocybe, whether they be Sanguinei or Cinnamomei.

a) Pigments peculiar to one or two species: they provide the /46
morphological differentiation from related species for which they are at times taken. Thus the non-anthraquinone type yellow pigment 13 from the foot of C. semisanguineus clearly separates that

species from C. phoeniceus (The bright fluorescence of the foot which enables one to easily distinguish between semisanguineus and phoeniceus is due to this pigment.) At the same time, the orange-yellow pigment 1 (emodine) and yellow 3 of malicorius differentiate it from conformis, even though they resemble each other.

b) Pigments characteristic of a section: for the sanguinei, we have pigments 3' and non-fluorescent 4, which we have not found among any Cinnamomei.

The light purple 4 is one of those primarily responsible for the platelet coloration of the Sanguinei. The yellow 3' seems less abundant, perhaps because it is pale; it is remarkable in that it turns blue-gray in the presence of ammonia.

The Cinnamomei are characterized by anthranol, the yellow pigment 2' responsible for the bright yellow masses R. Kühner observed after a long time among numerous Cinnamomei; it is however missing in two species, malicorius and olivaceofuscus; but it is always absent from the sanguinei. Other specific pigments of this section are the fluorescent orange pigment 7, pink 8 and yellow 9. None of these pigments appear in the Sanguinei although they can be found in most Cinnamomei, perhaps most of all the orange 7.

c) Pigments characteristic of a sub-genus: there are two fluorescent pigments, pink 5 and yellow 6. This pigment 6 is found in the subgenera of the Cortinaria other than Dermocybe, while until now pink 5 has not been discerned but in Dermocybe, where it seems to be one of the most general characteristics. C. olivaceofuscus is the only species in which we were unable to find it.

Moreover, one notes that these pigments common to the two sections have an average Rf, intermediate between the higher Rf of the pigments characteristic of Sanguinei and the lower Rf of the pigments characteristic of the Cinnamomei.

If one considers that the yellow pigment 6, or yellow pigments of very close Rf values, reappear among the Cortinaria other than Dermocybe, one could conclude that the differing specific pigments of Sanguinei and Cinnamomei derive from pigments of type 6.

/47

## SUB-GENUS HYDROCYBE FRIES

### Botanical Introduction

Few of the Hydrocybe show yellow or red coloration; however, the species of the section Miniatopoda have a red mycelium on the base of the foot. We studied one species of Miniatopoda: C.bulliardi Fr. ex Pers.

R. Kühner and H. Romagnesi (1953) have stated that C. cinnabrinus Fr., grouped by these authors among the Dermocybe due to its similarity to sanguineus, differs from the latter by its hygrophane pileus. For this characteristic it could be considered as a Hydrocybe.

On the other hand, R. Kühner observed the the foot color of cinnabrinus is very close to that of the base of bulliardi, for instance K 101 and 106*. If this similarity in coloration were to translate also into a similarity of constitution of the pigments, then the transfer of cinnabrinus from the Dermocybe to the Hydrocybe would be augmented. The particulars for this point are given below.

### Experimental Section

#### 1. STUDY OF THE PIGMENTS.

* K = Klincksieck and Valette. Color code, Paris, Paul Klincksieck editor.

1.1. EXTRACTION

The extraction of pigments from whole cinnabrinus carpophores and from the bases of the foot of bulliardi is accomplished with boiling alcohol on fresh material, for the reasons given in the paragraph dedicated to Dermocybe. We have also resorted to chloroform which extracts pigment I selectively.

1.2. CHROMATOGRAPHIC ANALYSES.

We have used primarily the chromatographic solvent chosen for the Dermocybe: iso-amylic alcohol - pyridine - water (30-20-15). We have also used the mixture butanol - acetic acid - water (25-6-25) as a second solvent for the comparison of species and, occasionally, Shibata's solvent I.

1.3. LIST OF PIGMENTS.

The pigments thus separated and their characteristics are given in Table V.

1.4. NATURE OF THE PIGMENTS. 48

The chromatographic analyses allow for the conclusion that the pigments are of the anthraquinone type. They also show (except for pigment I) that these anthraquinones are of a distinctly hydrophilic character, as opposed to the Dermocybe anthraquinones. However, none of the four pigments separated correspond to any of the pigments of Dermocybe.

Pigment I, which in the solvent iso-amylic alcohol - pyridine - water has the same Rf as emodine, could not be identified with it, since in Shibata's solvent I it migrates much less. Besides, it is not soluble in carbonates, which implies that all the hydroxyls are in α position.

TABLE V

SUB-GENUS HYDROCYBE. LIST OF PIGMENTS

(Chromatography in iso-amylic alcohol - pyridine - water.)

| | Rf | Aspect and colored reactions | | | |
|---|---|---|---|---|---|
| | | visible | U.V. | ammonia | magnesium acetate |
| I | 0.85 | yellow | citrine-yellow | pink | orange-yellow |
| II | 0.60 | yellow | dull orange | pink | orange-yellow |
| III | 0.40 | pink | orange-yellow | light purple | light purple |
| IV | 0.16 | bright pink | very luminous yellow | light purple | purple |

In the same manner, pigment III could not be identified with the pink 5 of the Dermocybe, as the comparative chromatograms showed; and if it partially reacted with zirconium nitrate, its fluorescent color is again very different.

For the same reasons pigment IV could not be likened to the pink 8 of the Dermocybe.

2. DISTRIBUTION OF PIGMENT ACCORDING TO SPECIES

See Table VI.

TABLE VI

DISTRIBUTION OF THE PIGMENTS

| | *cinnabarinus* | *bulliardi* |
|---|---|---|
| I | ** | ** |
| II | *** | * |
| III | *** | ** |
| IV | **** | **** |

Microtopography of the pigmentation

The red pigments are vacuolar in bulliardi and cinnabrinus; in particular, a red vacuolar pigment is observed in the pileus and the platelet stroma in cinnabrinus. With respect to the yellow pigments, R. Kühner has observed in several deep hyphae of the stem wall one, or at times two, or more golden spherical masses of blurred or spiked outline. We identified these masses as pigment I, distinguished by its lipophile character (solubility in chloroform.)

Taxonomic interest of the study of pigments

According to our findings, C. cinnabrinus does not have any of the pigments characteristic of Sanguinei, or of Dermocybe; on the other hand, the resemblance between the pigments of this species and those of bulliardi is striking and would seemingly allow us to place cinnabrinus with bulliardi, considering them Hydrocybe of the section Miniatopoda in which the red pigmentation pervades the whole carpophore, rather than being limited to the base of the carpophore. It is interesting to observe that an important

morphological characteristic in the classification of the Cortinaria (their hygrophane character) supports this similarity.

Let us say, however, that even if none of the pigments of the Dermocybe are found in bulliardi and cinnabrinus, we are dealing in all of these species with pigments of the same chemical family (anthraquinone pigments), reasonably similar due to certain structural characteristics, such as the presence of a -COOH substitute.

## SUB-GENUS PHLEGMACIUM FRIES

/50/

### Botanical Introduction

We studied four species with edged roots (section Scauri): C.elegantior sensu Moser, C. orichalceus Batsch ex Fr. sensu Moser, C. odorifer Britz., C. atrovirens Kalchbr., and two species without edged roots (section Cliduchi): C. percomis Fr. and C. nanceiensis R. Maire.

We established that our atrovirens is the species in which the pulp turns olive colored under the action of sodium hydroxide; this differentiates it very clearly from prasinus, the pulp of which turns red with that reagent. With respect to C. orichalceus, it is to be taken in the sense of Moser and R. Kühner. It agrees with the description given by the latter (R. Kühner, 1960)

### Experimental Section

1. STUDY OF THE PIGMENTS.

1.1. EXTRACTION.

As for the other sub-genera, we used boiling alcohol. Some of the pigments of odorifer, orichalceus, nanceiensis and percomis

TABLE VII

SUB-GENUS PHLEGMACIUM. CHARACTERIZATION OF ANTHRANOLS

| Chloroform extracts | Sodium hydroxide | Sulfuric acid | Mecke's reagent |
|---|---|---|---|
| elegantior | yellow | red | green ring darkening then brown |
| odorifer | yellow to orange-yellow | red | green ring darkening then black |
| atrovirens | gold yellow | red | green ring darkening then brown |
| nanceiensis | yellow | red to red-dish brown | green ring then brown-ish green |
| percomis | yellow | red to red-dish brown | green ring then brown-ish green |

are more or less altered by boiling alcohol or cold alcohol: particularly, a purple precipitate appears that is very difficult to dissolve (in dioxane for odorifer and orichalceus, and in chloroform for nanceiensis and percomis). It is for this reason that we also went to chloroform extractions, which yield stable, yellow solutions.

## 1.2. CHROMATOGRAPHIC ANALYSES AND NATURE OF THE PIGMENTS.

### 1.2.1. Alcoholic extracts

Chromatographic analyses in the solvent iso-amylic alcohol - pyridine - water revealed, for all species, spots that are more or less violet to brownish-yellow, due to the modification of initially yellow pigments; their variable Rf is generally comprised

between 0.9 and 1.

Besides, several pigments showing hydroxyanthraquinone reactions are separated in the case of elegantior and odorifer. For elegantior, two yellow pigments (Rf 0.35 and 0.25) and one orange pigment (Rf 0.02), are found only in the bulb of the foot; for odorifer, a yellow pigment (Rf 0.22). The latter is reminiscent of pigment 6 of the Dermocybe, for its color, its fluorescence and its reaction with magnesium acetate; they differ slightly in their Rf value. With respect to the yellow pigment of Rf 0.35 of elegantior, /51 comparative chromatographic analysis identifies it perfectly with pigment 6, characteristic of the Dermocybe.

1.2.2. Chloroform extracts.

1.2.2.1. Characterization of anthranols.

See Table VII for the action of various reagents.

Oxidation to anthraquinones.

The chloroformic extract of the five species shown in Table VII is shaken with a normal sodium hydroxide solution. The alkaline solution is colored yellow. We then add some approximately 3% hydrogen peroxide solution. The solutions are mixed in a boiling water bath for several minutes. Their coloration becomes purplish red to violet red and, after acidification, the pigments pass into ether and are taken up again in 95°ethanol. In the presence of magnesium acetate and sodium hydroxide they show the changes characteristic of anthraquinone (notably, purple to violet with magnesium acetate).

1.2.2.2. Chromatographic analysis

In the solvent 30-20-15 (iso-amylic alcohol - pyridine - water)

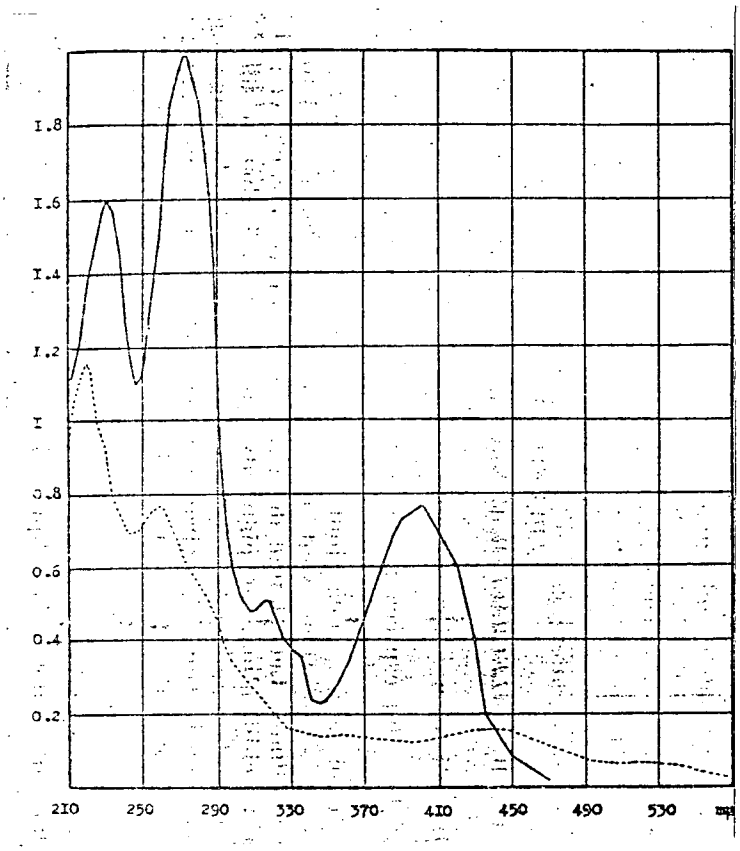



Figure 8. Absorption curve of anthranol from C. percomis (95°ethanol). Dotted: after oxidation to anthraquinone.

as well as in Partridge's classical solvent (butanol - acetic acid - water (40-10-50), the anthranols move with the solvent front. To compare the different species we used the mixture acetic acid - water (30 %), in which the extracts divided into two fractions: one brownish of Rf 0, the other yellow to brown yellow with the Rf varying between 0.7 (percomis - nanceiensis) to 0.6 (atrovirens - elegantior - odorifer - orichalceus). We observed that the yellow pigments (Rf 0.7) of percomis and nanceiensis had a yellow to greenish-yellow fluorescence under Wood's light. /53

We point out the particular instability of the anthranols from odorifer and orichalceus, which became oxidized in the course of

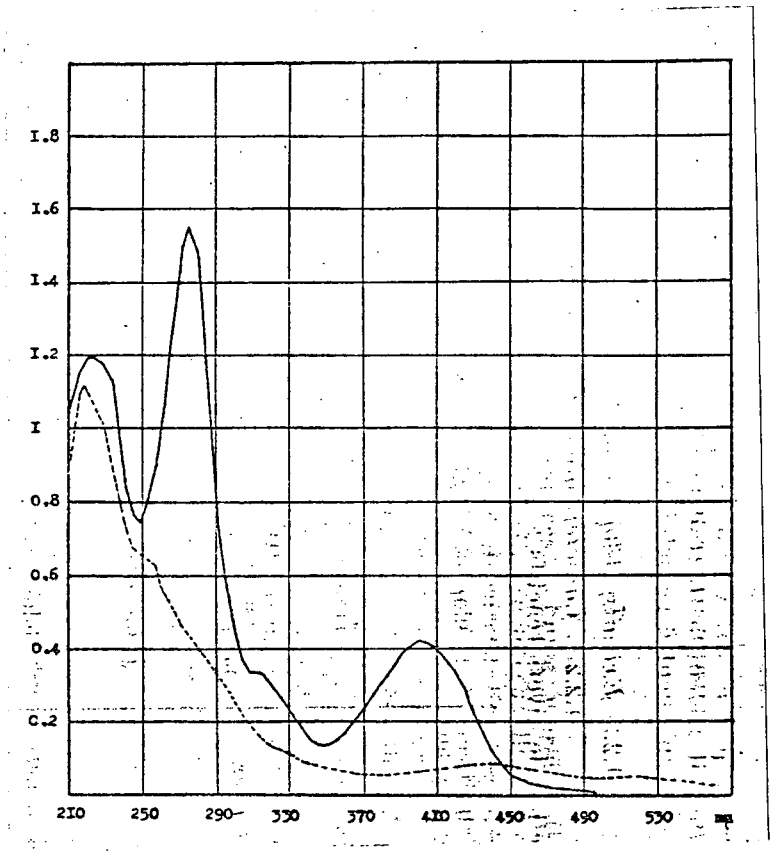



Figure 9. Absorption curve of anthranol from C. nanceiensis (95°ethanol).Dotted: after oxidation to anthraquinone

the chromatography. The others did not show modification until after one or two weeks.

1.2.2.3. Spectrophotometry.

The absorption curves for the anthranoles from percomis and the anthranoles form nanceiensis (pigments purified by chromatography) are very similar (Figures 8 and 9):

percomis λM ethanol: 230 - 272 - 315 - 400 mμ
nanceiensis λM ethanol: 222 - 275 ~ 315 - 400 mμ

Microtopography of the pigmentation

In our reporting on observations made by R. Kühner on the pigmentation of the Phlegmacia (R. Kühner, 1960 and unpublished notes on percomis, nanceiensis, atrovirens and odorifer) we confirmed that the Phlegmacia have a yellow to green-yellow extra or epicellular pigment, in the pulp or the stroma of the platelets:

C. atrovirens: enormous refractive masses of a beautiful green-yellow in the pulp of the foot, plus small ones in the pileic pulp, producing oils of the same color: small drops or yellowish plates very hard to find in the stroma of the platelets.

C. odorifer: the pileic pulp and the stroma of the platelets has yellow, very small extra-epicellular granulations; old basidia had bright yellow ones.

C.orichalceus: in the stroma of the platelets, very fine intercellular granules, or covering the surface of hyphae.

C. percomis: bright yellow pigmentary granulations, inter and epicellular in the platelets' stroma, the pileic pulp and the back of the platelets.

C. nanceiensis: on the stem, as golden citrine pigments forming irregular masses among the hyphae or incrusted in the membrane. The pigment is visible both in the pileic pulp and the platelets' stroma.

C. elegantior: brownish yellow masses of large size but well dispersed. Small extra-epicellular yellow masses, often retreating and reappearing with age in the midsection. /54

We personally observed some yellow granules in odorifer and some basidia of yellow content, which appear under the microscope with

an orange yellow colored fluorescence; for percomis, the bright yellow granules have an orange fluorescence, while the very small granules in certain basidia are packed; for nanceiensis, both the bands and the very small granules incrusted in the membrane show an orange-yellow fluorescence.

There is every reason to believe that the fluorescence that the pulp of this species shows under "Wood's light" is due at least in part to those yellow extra-cellular granules observed by R. Kühner. These granules are identifiable with anthranols, lipophile pigments characterized by chemical analyses.

It would be interesting to investigate the possible presence, in elegantior or odorifer, of a vacuolar pigment corresponding to the anthraquinone pigments found among these species.

Taxonomic interest in the study of pigments

The principal pigments of the species studied are the anthranoles. As the anthranoles of the Cinnamomei, they are located at the level of the yellow intercellular masses, or the granules or droplets.

C. percomis and nanceiensis probably have the same anthranol. In elegantior and odorifer one again finds the anthraquinone pigment 6 (or a very similar pigment), common to all Dermocybe.

## SUB-GENUS CORTINARIUS (= INOLOMA Fr.)

### SECTION OLIVACEOAURATI LANGE
(= Olivascentes Kühner and Romagnesi)

### Botanical introduction

This section was placed among the Dermocybe by Fries, who differentiated it from our Sanguinei and Cinnamomei by the olive color of its species. This distinction lacks sharpness, as there exist some olive colored Cinnamomei. Much greater differences provide a delimitation for this section: the spores are subglobular, the pileic covering is made up of much greater hypha, and to this one must add some characteristics of a chemical nature. The pulp of this species shows a characteristic reaction: it becomes bright yellow with silver nitrate (this reaction was discovered by R. Henry (1937) on cotoneus, and found again by R. Kühner (1960) on melanotus and venetus). We have confirmed that all these species show, under Wood's light, a beautiful vivid yellow fluorescence of the foot, platelets and the pulp (M. Gabriel 1962). Elsewhere, Singer (1962) discovered, following microscopic studies, the characteristic presence of a fluorescent pigment: "pigment pale yellow in outer layers covered by an olive green pigment which appeared extremely brightly and brilliantly chrome yellow to emerald green under the fluorescence microscope".

Singer transferred the Olivaceoaurati to the sub-genus Inoloma which, for reasons of rules of nomenclature, are called today Cortinarius.

### Species studied:

C. cotoneus Fr. sensu Quelet, C. venetus Fries and C. melanotus Kalchbr.

These species are taken in the sense of R. Kühner (1960).

## Experimental Section

### 1. STUDY OF THE PIGMENTS.

#### 1.1. EXTRACTION.

We performed our extractions in boiling ethanol. The yellow and brownish yellow colored extracts showed the bright fluorescence of the carpophores.

#### 1.2. CHROMATOGRAPHIC ANALYSES. /56

The analysis showed the existence of numerous pigments; three among these, simultaneously present in significant amounts in each of the species studied, are designated as A, B and C, while the others are α, β, γ and δ.

The majority of these pigments do not migrate in the solvent used for the chromatography of the anthraquinones from the Dermocybe (iso-amylic alcohol - pyridine - water); however, in the case of cotoneus this solvent clearly evidenced two of them, γ and δ, yellow pigments of Rf 0.2 and 0.3; pigment δ was the most abundant.

With very aqueous solvents, acidic or alkaline, a good separation is possible; in particular: water - acetic acid (85-15) and 4% sodium citrate saturated with iso-amylic alcohol. Due to its alkaline pH the latter had the advantage of brightening the color of the spots. We used the water - acetic acid (85-15) solvent when elutions were necessary.

In each of the extracts the three pigments A, B and C are thus

TABLE VIII

SECTION OLIVACEOAURATI. LIST OF THE PIGMENTS.
(Chromatography in citrate solvent.)

| | Rf | Aspect and colored reactions | | | |
|---|---|---|---|---|---|
| | | visible | U.V. | ammonia visible | ammonia U.V. |
| α | 0 | light brown | cream color | brown | creme color |
| β | 0.01 | pale red | reddish | red | reddish |
| A | 0.09 | pale gr. yellow | luminous grn.-yellow | blue | blue |
| γ | 0.10 | pale red | reddish | red | reddish |
| δ | 0.20 | pale red | reddish | red | reddish |
| B | 0.36 | citrin yellow | very bright yellow | intensif. yellow | blue |
| C | 0.53 | pale yllw. orange | dull green | orange yellow | greenish |

separated; besides, from the extracts of whole carpophores of cotoneus and melanotus we recovered the pigments γ and δ previously found; due to the pH of the solvent their color is pale red. In the extracts of platelets of venetus and melanotus we found a third pigment, β, of the same color and last, a light brown pigment α, but only in the carpophores of melanotus.

## 1.3. LIST OF PIGMENTS

The list of the pigments thus separated in citrate solvent, and their aspect before and after the action of certain reagents is

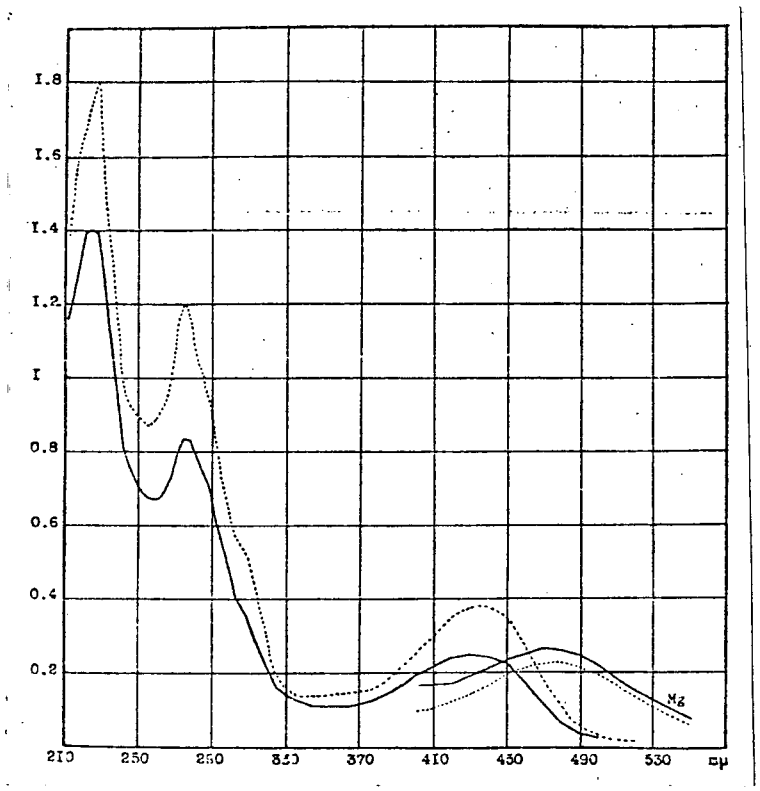



Figure 10. Absorption curve of the anthraquinone type pigment of C. cotoneus (solid line) and of the anthraquinone 6 of the Dermocybe (dotted line, in 95°ethanol, Mg: in the presence of Mg acetate.

given in Table VIII. We point out that pigment B turns intensely yellow with silver nitrate.

## 1.4. STRUCTURE OF THE PIGMENTS.

### 1.4.1. Anthraquinone type pigments

For its Rf (in comparative chromatography), its color in the visible region, in the U.V. and after the action of magnesium acetate and ammonia vapors, the pigment δ has been identified with the yellow pigment 6 of the Dermocybe. Spectrophotometry is in

agreement with this conclusion: the absorption curves in the U.V. and the visible are identical (see Figure 10, page 65)

The other pigments, β and γ, much less abundant, are probably also of anthraquinone type.

1.4.2. Xanthone type pigments /57

1.4.2.1. Separation and crystallization

The alcoholic extract is precipitated with neutral lead acetate. The precipitate is centrifuged, washed and taken up in 2N $H_2SO_4$. The lead sulfate is eliminated by recentrifuging. The acid solution obtained is diluted and then extracted several times with n-butanol. The butanol fraction, left at ambient temperature, produces yellow needles, which under the fluorescence microscope show a vivid yellow color. We proved by means of paper chromatography that the crystals are basically pigment B; after a second crystallization there still is some trace of the green-yellow pigment A left.

Properties of the crystals: /58

Melting point: over 350°C

Solubility: the crystals are easily water soluble, poorly soluble in ethanol and butanol, and insoluble in benzene, chloroform and ether.

Action of acids and bases: in concentrated $H_2SO_4$, $HNO_3$ and HCl, the crystals turn red, dissolve more or less and yield a yellow solution. In an alkaline bath, the crystals turn red and then dissolve; the solution rapidly becomes orange-yellow. An aqueous or alcoholic solution of the crystals, of a pale yellow color, turns dark yellow in the presence of sodium hydroxide.

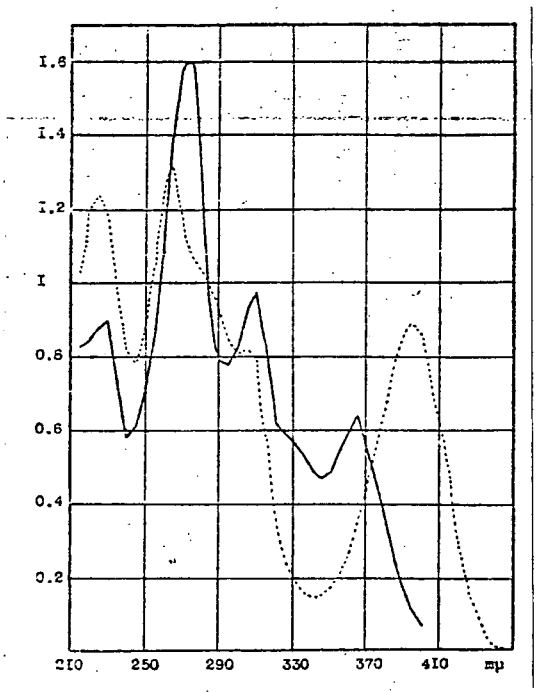



Figure 11. Absorption curve of the xanthone type pigment B (glycoside) in distilled water; dotted: in 0.02N sodium hydroxide

U.V. and visible spectrophotometry: λM water: 230 - 272 - 309 - 366 mμ (Figure 11, above).

1.4.2.2. Acid microhydrolysis

We have carried out many hydrolyses, starting either with crystals or with the eluates obtained chromatographically. Hydrolysis is performed in sealed tubes at 100° for 16 hours in 1N HCl or 4 hours in 3N $H_2SO_4$; each time a brown-yellow precipitate is formed. This precipitate, isolated and subjected to chromatography in citrate solvent, is shown for the most part to be identical with pigment A.

The aqueous fraction, once neutralized, is also subjected to chromatography, to investigate for sugars, in the following three

solvents: butanol - acetic acid - water (40-10-50), water saturated phenol, and ethyl acetate - pyridine - water (20-10-20). We thus have proof of the presence of glucose, in the case of the two hydrolyses. Moreover, in the case of the hydrochloric hydrolysis, a relatively important supplementary product appears, of an Rf lower than that of glucose in the solvent ethyl acetate - pyridine - water, which turns brown with aniline phthalate; after the sulfuric hydrolysis we obtain a compound that seems similar to xylose for its Rf and its pink to reddish brown coloration after the action of aniline phthalate.

After hydrolysis in 1N $H_2SO_4$ for an hour, 470 mg of crystals gave 170 mg of aglycone, which represents 300 mg of sugars, or about 60 % of the weight of the glycoside (the content seems rather high but we point out that the filtrate was still colored.) Under these conditions of hydrolysis, the "sugar fraction" showed nothing but glucose.

Hence pigment B exists in glycosidic form.

1.4.2.3. Characterization of the aglycone. /61

Melting point: As for the glycoside, the melting point is higher than 350°C.

Solubility:

Poorly soluble in organic solvents, insoluble in water, chloroform, ether; slightly soluble in ethanol; the best solvent is methyl alcohol.

U.V. and visible spectrophotometry:

The U.V. spectrum proved very interesting in that it is typical of a xanthone type compound.

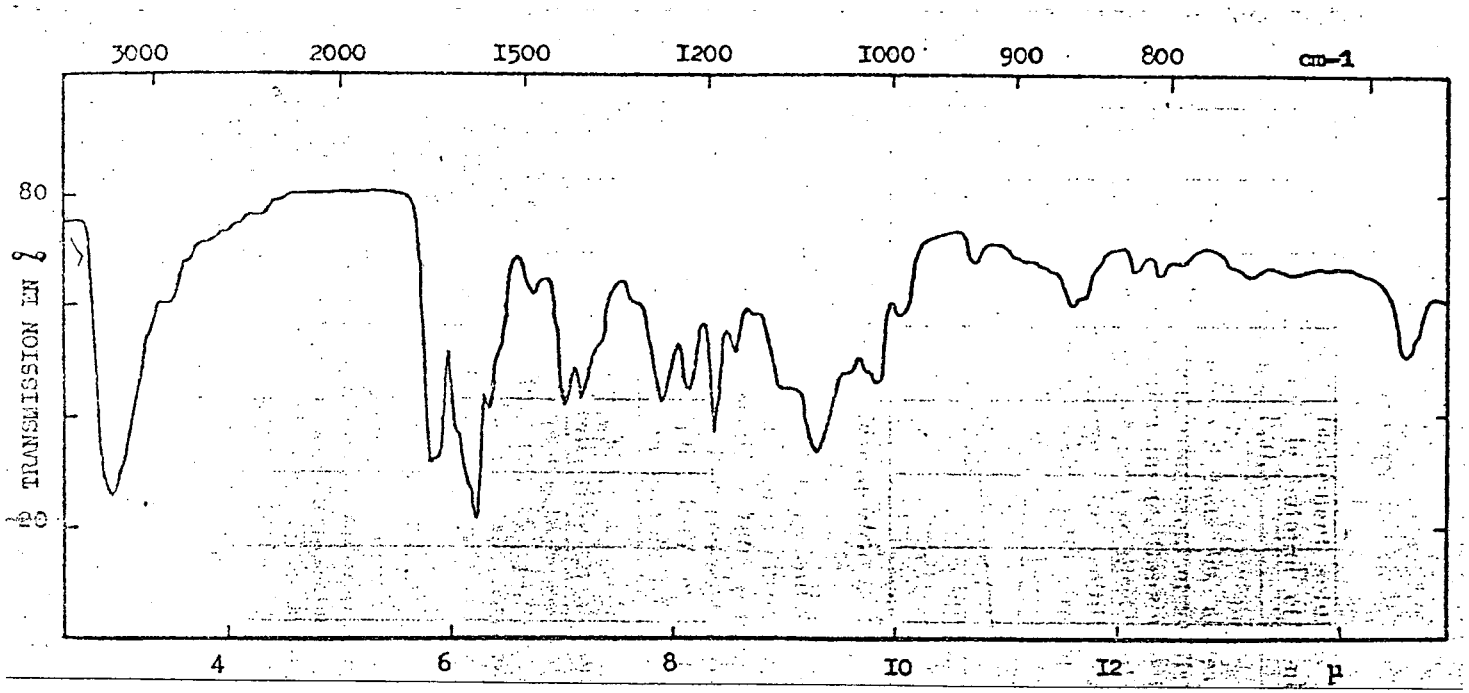



Figure 12. I.R. Spectrum of the xanthone type pigment B (glycoside) (KBr pellet)

TABLE IX

SECTION OLIVACEOAURATI. DISTRIBUTION OF PIGMENTS

| | *venetus* | *melanotus* | *coloneus* |
|---|---|---|---|
| α | | ** | |
| β | * | * | |
| A | ** | ** | ** |
| γ | | | * |
| δ | | * | ** |
| B | **** | **** | **** |
| C | ** | ** | ** |

λM ethanol: 230 - 272 - 307 - 366 mμ.

We have not observed any bathochrome displacement of the 366 mμ maximum after reaction with aluminum chloride; this implies that there is no -OH in positions 1 or 8 (the chelation of $Al^{+3}$ involves this hydroxyl group).

I.R. Spectrophotometry*:

The I.R. spectrum is compatible with the hypothesis of a xanthonic compound. We point out two bands indicative of carbonyls:

* We used the Perkin-Elmer 21 apparatus.

the free -C = O : 1718 $cm^{-1}$, the conjugated -C = O, with a phenyl: 1684 $cm^{-1}$;

some phenyl bands: 1634, 1490 $cm^{-1}$

some phenolic bands: 3030, 1385, 1193 $cm^{-1}$.

We point out that with pigment B we obtained the 1074 $cm^{-1}$ band, characteristic of glycosides. (Figure 12.)

Alkaline fusion:

The alkaline fusion according to A. Maurice's method yielded two phenolic compounds which we have not been able to identify, but which are not phloroglucine or orcine, two phenols shown in the course of the alkaline fusion of some xanthones.

In summary, we have then isolated a xanthone that does not seem known, because all those indicated in the literature had a -OH group in 1 or in 8.

## 2. DISTRIBUTION OF PIGMENTS ACCORDING TO SPECIES.

(See Table IX, page 70)

Microtopography of the pigmentation

/62

The chemical studies have shown that the majority of the separated pigments are of a distinctly hydrophile character (glycoside, anthraquinone 6); it is to be expected that the pigmentation of the Olivaceoaurati is essentially vacuolar. This is confirmed by observations made by R. Kühner, who particularly noted the vacuolar colorations at the level of the carpophore surface (R.Kühner 1960). Thus, in venetus the hyphae of the universal veil contain vacuola vividly colored in citrine or olive-yellow, which turn

copper red with ammonia; they probably contain an anthraquinone type pigment. In melanotus the hyphae of the pileic covering at the base of the universal veil or veil hypha, have yellow colored vacuola; with respect to the sepia brown coloration characteristic of the universal veil of this species, it is vacuolar and corresponds to pigment α, light brown and found only in this mushroom. In cotoneus, the observed colors are less intense and the pileic surface hyphae are here light yellow to very pale olive. We have personally observed, in the pileic cup of venetus, some very fluorescent vacuolae, a fluorescence probably due to the glycoside.

R. Kühner never found large, bright yellow masses comparable to /63
those of the Cinnamomei, in the platelet stroma of the Olivaceoaurata; however, in one instance he observed, near the edge of the platelets of venetus, some pale olive colored masses, of a small volume, that could correspond to the aglycone (pigment A). With respect to old basidia, they seemed to him filled with a light yellow or slightly brown pigment. For this same species we observed that the basidia have a very bright yellow fluorescence.

## Taxonomic interest of the study of pigments

The three species studied, which represent the majority of the species in this section, show a great pigmentary similarity. It is interesting to confirm the presence of anthraquinone type pigments in these species, notably to find in one of them an anthraquinone identified with the yellow pigment 6 of the Dermocybe.

But the Olivaceoaurati are distinguished by a pigment of a xanthone nature, pigment B, to which we attribute the yellow reaction with silver nitrate, that the pulp of all three of the species studied show. We recall that up until now hydroxyxanthones were not known except in lower mushrooms. On the other hand, we

stress that this pigment B is present as a glycoside. To our knowledge, it had not been established with any certainty that glycosidic pigments exist both in lower and higher mushrooms*.

We point out that the two structural groups xanthones and anthraquinones are not as far removed as one might think. Gatenbeck (1960), studying the biogenesis of anthraquinones in imperfect mushrooms, cites xanthones as either an intermediate product or an oxidation product.

* Kögl thought that the muscarafine of Amanita muscaria was in glycosidic form (Kögl and Ernleben, 1930)

CONCLUSION



During the six years of research on the pigments of the Boletus and the Cortinaria, we have collected and studied almost all the Boletaceae, Gomphidiaceae and Paxillaceae in the French flora (41 species) and a certain number of Cortinaria (21 species) distributed among four different sub-genera: Dermocybe (were we obtained most species), Hydrocybe, Phlegmacia and Cortinarius sensu stricto (= Inoloma).

One part of our presentation is concerned with the enumeration of the chemical characteristics of the pigments; the other, with the systematic interest these pigments have.

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For the Boletus and related species, the chromatographic analysis of extracts has allowed us to clearly separate and to characterize boletol, a known anthraquinone type pigment, and to prove that in some Boletus and all the Gomphidia a chemically similar pigment exists, that reacts in line with phenyloxidase and which we have called pseudoboletol.

We have also shown other pigments, at times quantitatively important, some of which are of an anthraquinone nature.

From the taxonomical point of view, the presence or absence of boletol corroborates the divisions of the systematicians: thus all the Boletus Dill. ex Fr. sensu stricto (= Tubiporus Karst.) contain boletol, even though one Leccinum S.F. Gray (= Krombholzia Karst.) does not contain it. Also, all the Xerocomus Quel. do contain boletol, and so does the gilled species Phylloporus rhodoxanthus Schw., grouped by systematicians with the Xerocomus.

We have called attention to the fact that X. parasiticus contains much more pseudoboletol than boletol.

The existence of pseudoboletol in all the species of Gomphidius reaffirms the closeness of this genus to the Boletus.

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In the case of the Cortinaria, some anthraquinones and some anthranoles have been found in all the sub-genera studied. In just the sub-genus Dermocybe we have enumerated 14 anthraquinones and 1 anthranol. On the other hand, in the sub-genus Phlegmacium the anthranoles are the principal pigments.

Besides emodine and dermocybine, we have been able to identify these pigments with known anthraquinones. We have characterized /66
them chromatographically, by spectrophotometry and by various chemical reactions, and have dealt with the problem of their structure.

In the Olivaceoaurati section of the sub-genus Cortinarius sensu stricto, we have also isolated a xanthonic pigment; this type of pigment had never been shown in the higher mushrooms. We have shown it to exist in a glycosidic form.

The problem of the distribution of these pigments in the different parts of the carpophora has been studied, as has that of their microscopic localization. The latter point has been presented in the paragraph "Microtopography" corresponding to the study of each sub-genus. Notably, we have attributed to an anthranol the characteristic pigmentary masses observed microscopically in a whole group of Dermocybe.

In a general way the results of our research agree with the divi-

sions of the systematicians. We have pointed out, however, that C. (Dermocybe) cinnabrinus is much closer to C. (Hydrocybe) bulliardi because of its pigments than it is to other Dermocybe.

Otherwise our results confirm the homogeneity of the genus Cortinarius. In fact, identical or similar pigments are found among the species with a yellow coloration of their pulp, their foot or their platelets, but otherwise quite different, for which reasons they are grouped in different sub-genera: we point out the anthraquinone type pigment "6" of the Dermocybe, Cortinarius (=Inoloma) and Phlegmacia, and the anthranoles found in the Dermocybe Cinnamomei and in Phlegmacium varieties, all of the Scauri section.

Some mycologists, like Moser in 1951, tend to look at Dermocybe in the strict sense, that is, the group centered around the Cinnamomei and some related species of the genus Gymnopilus Karst. (Fulvidula Romagnesi.) Moser observed that the species of this sub-genus are much closer to the genus Gymnopilus Karst., for their color, their reactions to bases and their spores, than they are to Phlegmacium, for instance, and the greater part of the other Cortinaria. After studying two Gymnopilus, G. hybridus and G. spectabilis, we extracted several pigments and we can say that we are not dealing with either anthraquinones or anthranoles. Thus this comparison has not been confirmed by chemical results.

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In conclusion, the study we have attempted of the pigments of certain Agaricales has permitted us to more closely define the similarities among families, genera, sub-genera and species. It has, on the other hand, shown the existence of numerous pigments in particular for which structural studies would notably enrich the knowledge of natural anthraquinones and xanthones.

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